NOVEL COMPOSITIONS FOR THE DELIVERY OF NEGATIVELY CHARGED MOLECULES

Field of the Invention

This patent application is a continuation of Beigelman *et al.,* USSN 09/120,520, filed July 21, 1998, entitled "NOVEL COMPOSITIONS FOR THE DELIVERY OF NEGATIVELY CHARGED MOLECULES", which claims priority from Beigelman *et al.,* USSN (60/053,517), filed July 23, 1997, entitled "NOVEL COMPOSITIONS FOR THE DELIVERY OF NEGATIVELY CHARGED MOLECULES" and Beigelman *et al.*, USSN (60/072,967), filed January 29, 1998, entitled "NOVEL COMPOSITIONS FOR THE DELIVERY OF NEGATIVELY CHARGED MOLECULES". These applications are hereby incorporated by reference herein in their entirety including the drawings.

Background of the Invention

The following is a brief description of the delivery of biopolymers. This summary is not meant to be complete but is provided only for understanding of the invention that follows. This summary is not an admission that all of the work described below is prior art to the claimed invention.

Trafficking of large, charged molecules into living cells is highly restricted by the complex membrane systems of the cell. Specific transporters allow the selective entry of nutrients or regulatory molecules, while excluding most exogenous molecules such as nucleic acids and proteins. The two major strategies for improving the transport of foreign nucleic acids into cells are the use of viral vectors or cationic lipids and related cytofectins. Viral vectors can be used to transfer genes efficiently into some cell types, but they cannot be used to introduce chemically synthesized molecules into cells. An alternative approach is to use delivery formulations incorporating cationic lipids, which interact with nucleic acids through one end and lipids or membrane systems through another (for a review see Felgner, 1990, *Advanced Drug Delivery Reviews,* 5,162-187;

Felgner 1993, *J. Liposome Res.,* 3,3-16). Synthetic nucleic acids as well as plasmids may be delivered using the cytofectins, although their utility is often limited by cell-type specificity, requirement for low serum during transfection, and toxicity.

Since the first description of liposomes in 1965, by Bangham (*J. Mol. Biol.* 13, 238-252), there has been a sustained interest and effort in the area of developing lipid-based carrier systems for the delivery of pharmaceutically active compounds. Liposomes are attractive drug carriers since they protect biological molecules from degradation while improving their cellular uptake.

One of the most commonly used classes of liposomes formulations for delivering polyanions (*e.g.,* DNA) are those that contain cationic lipids. Lipid aggregates can be formed with macromolecules using cationic lipids alone or including other lipids and amphiphiles such as phosphatidylethanolamine. It is well known in the art that both the composition of the lipid formulation as well as its method of preparation have effect on the structure and size of the resultant anionic macromolecule-cationic lipid aggregate. These factors can be modulated to optimize delivery of polyanions to specific cell types *in vitro* and *in vivo.* The use of cationic lipids for cellular delivery of biopolymers have several advantages. The encapsulation of anionic compounds using cationic lipids is essentially quantitative due to electrostatic interaction. In addition, it is believed that the cationic lipids interact with the negatively charged cell membranes initiating cellular membrane transport (Akhtar *et al.,* 1992, *Trends Cell Bio.*, 2, 139; Xu *et al.,* 1996, *Biochemistry* 35, 5616).

The transmembrane movement of negatively charged molecules such as nucleic acids may therefore be markedly improved by coadministration with cationic lipids or other permeability enhancers (Bennett *et al.,* 1992 *Mol. Pharmacol.*, 41, 1023-33; Capaccioli *et al.,* 1993, *BBRC,* 197,818-25; Ramila *et al.,* 1993 *J. Biol. Chem.,* 268,16087-16090; Stewar *et al.,* 1992, *Human Gene Therapy*, 3, 267-275). Since the introduction of the cationic lipid DOTMA and its liposomal formulation Lipofectin® (Felgner *et al.,* 1987, *PNAS* 84, 7413-7417; Eppstein *et al.,* U.S. Patent number 4,897,355), a number of other lipid-based delivery agents have been described primarily for transfecting mammalian cells with plasmids or antisense molecules (Rose, U. S. Patent No. 5,279,833; Eppand *et al.* U. S. Patent No. 5,283,185; Gebeyehu *et al.,* U. S.

Patent No. 5,334,761; Nantz *et al.,* U. S. Patent No. 5,527,928; Bailey *et al.,* U. S. Patent No. 5,552,155; Jesse, U.S. Patent No. 5,578,475). However, each formulation is of limited utility because it can deliver plasmids into some but not all cell types, usually in the absence of serum (Bailey *et al.,* 1997, *Biochemistry,* 36, 1628). Concentrations (charge and/or mass ratios) that are suitable for plasmid delivery (~5,000 to 10,000 bases in size) are generally not effective for oligonucleotides such as synthetic ribozymes or antisense molecules (~10 to 50 bases). Also, recent studies indicate that optimal delivery conditions for antisense oligonucleotides and ribozymes are different, even in the same cell type. However, the number of available delivery vehicles that may be utilized in the screening procedure is highly limited, and there continues to be a need to develop transporters that can enhance nucleic acid entry into many types of cells.

Eppstein *et al.,* U.S. Patent No. 5,208,036, disclose a liposome, LIPOFECTIN™, that contains an amphipathic molecule having a positively charged choline head group (water soluble) attached to a diacyl glycerol group (water insoluble). Promega (Wisconsin) markets another cationic lipid, TRANSFECTAM™, which can help introduce nucleic acid into a cell.

Wagner *et al.,* 1991, *Proc. Nat. Acad. Sci. USA* 88, 4255; Cotten *et al.,* 1990, *Proc. Nat. Acad. Sci. USA* 87, 4033; Zenke *et al.,* 1990, *Proc. Nat. Acad. Sci. USA* 87, 3655; and Wagner *et al.,* 1990, *Proc. Nat. Acad. Sci. USA* 87, 3410, describe transferrin-polycation conjugates which may enhance uptake of DNA into cells. They also describe the feature of a receptor-mediated endocytosis of transferrin-polycation conjugates to introduce DNA into hematopoietic cells.

Wu *et al.,* 1991, *J. Biol. Chem.* 266, 14338, describe *in vivo* receptor-mediated gene delivery in which an asialoglycoprotein-polycation conjugate consisting of asialoorosomucoid is coupled to poly-L-lysine. A soluble DNA complex was formed capable of specifically targeting hepatocytes via asialoglycoprotein receptors present on the cells.

Clark *et al.,* International PCT Publication No. WO 91/18012, describe cell internalizable covalently bonded conjugates having an "intracellularly cleavable linkage" such as a "disulfide cleavable linkage" or an enzyme labile ester linkage.

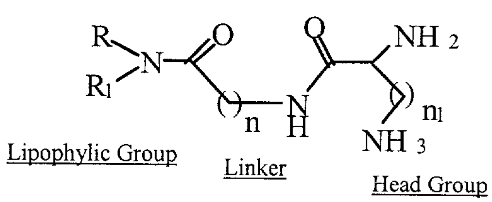
Brigham, U.S. Patent No. 5,676,954 describes a method for the expression of nucleic acid following transfection into a target organ consisting of mammalian cells.

The references cited above are distinct from the presently claimed invention since they do not disclose and/or contemplate the delivery vehicles of the instant invention.

Summary of the Invention

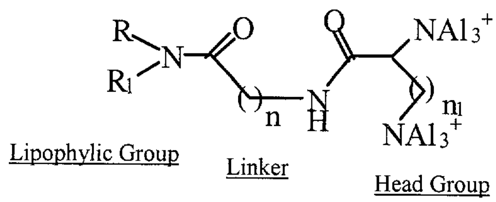
This invention features lipid-based compositions, such as cationic lipid-based compositions, that facilitate delivery of molecules into a biological system, for example into cells such as mammalian cells. The present invention discloses the design, synthesis, and cellular testing of novel agents for the delivery of various molecules, for example nucleic acids, polynucleotides, oligonucleotides, and/or negatively charged molecules, *in vitro* and *in vivo*. Also disclosed are screening procedures for identifying the optimal delivery vehicles for any given nucleic acid and cell type. In general, the transporters or cytofectins described here are designed to be used either individually or as part of a multi-component system. Examples of such multi-component systems comprising lipid compounds of the invention are shown in Tables II,VI, VII, VIII, and IX. The lipid compounds of the invention generally shown in Figure 1 and Table X, are expected to improve delivery of nucleic acids, polynucleotides, oligonucleotides, and/or negatively charged molecules, into a number of cell types originating from different tissues, in the presence or absence of serum.

In one embodiment, the invention features a cationic lipid having the formula I:



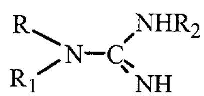
wherein, n is 1, 2 or 3 carbon atoms; n_1 is 2, 3, 4 or 5 carbon atoms; R and R_1 independently represent C12-C22 alkyl chain which are saturated or unsaturated, wherein the unsaturation is represented by 1-4 double bonds; and R_2 and R_3 are independently H, acyl, alkyl, carboxamidine, aryl, acyl, substituted carboxamidine, polyethylene glycol (PEG) or a combination thereof.

In another embodiment, the invention features a cationic lipid having the formula II:



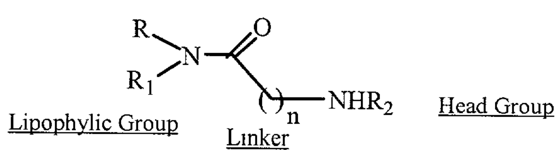
wherein, n is 1, 2 or 3 carbon atoms; n_1 is 2, 3, 4 or 5 carbon atoms; R and R_1 independently represent C12-C22 alkyl chain which are saturated or unsaturated, wherein the unsaturation is represented by 1-4 double bonds; and Alk represents methyl, hydroxyalkyl (*e.g.*, hydroxymethyl and hydroxyalkyl) or a combination thereof.

In another embodiment the invention features a cationic lipid having the formula III:

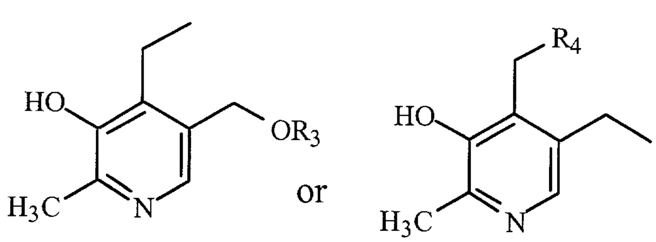


wherein, R and R_1 independently represent C12-C22 alkyl chain which are saturated or unsaturated, wherein the unsaturation is represented by 1-4 double bonds; and R_2 is H, PEG, acyl or alkyl.

In another embodiment the invention features a cationic lipid having the formula IV:

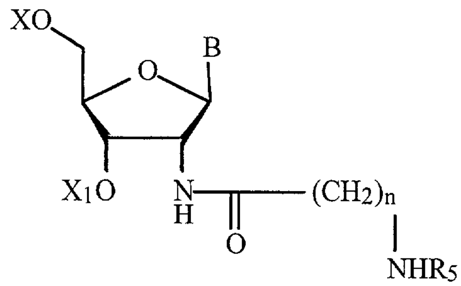


wherein, n is 1-6 carbon atoms; R and R_1 independently represent C12-C22 alkyl chain which are saturated or unsaturated, wherein the unsaturation is represented by 1-4 double bonds; and R_2 is H, carboxamidine, alkyl, acyl, aryl, PEG, substituted carboxamidine



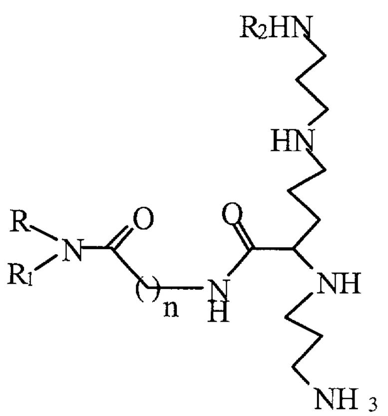
where R_3 is H, or PO_3H_2 and R_4 is OH, NH_2 or =O..

In one embodiment the invention features a cationic lipid having the formula V:

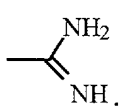


wherein, n is 1-6 carbon atoms; X and X_1 independently represent C12-C22 alkyl chain which are saturated or unsaturated, wherein the unsaturation is represented by 1-4 double bonds; B is a nucleic acid base or H; and R_5 is H, PEG, or carboxamidine.

In another embodiment the invention features a cationic lipid having the formula VI:



wherein, n is 1, 2 or 3 carbon atoms; R and R_1 independently represent C12-C22 alkyl chain which are saturated or unsaturated, wherein the unsaturation is represented by 1-4 double bonds; and R_2 and R_3 is independently H, polyethylene glycol (PEG) or



In the above referenced formulae, N, O, and H are Nitrogen, Oxygen, and Hydrogen, according to the abbreviations well-known in the art.

In another embodiment the invention features a cationic lipid having the formula VII:

R_6-L_1-Cholesterol

wherein, R_6 is selected from the group consisting of arginyl methyl ester, arginyl amide, homoarginyl methyl ester, homoarginyl amide, ornithine methyl ester, ornithine

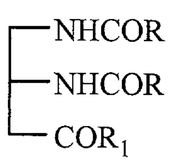
amide, lysyl methyl ester, lysyl amide, triethylenetetramine (TREN), *N,N'*-di-carboxamidine TREN, *N*-benzyl histidyl methyl ester, pyridoxyl and aminopropylimidazole. L_1 is a linker represented by R_7PO2, wherein R_7 is H, CH_3, or CH_2CH_3. Examples of this group of compounds are: PH55933, PH55938, PH55939, PH55941, PH55942, PH55943 and PH55945.

In another embodiment the invention features a cationic lipid having the formula VIII:

R_8-L_2-Cholesterol

wherein, R_8 is selected from the group consisting of arginyl, N-Boc arginyl, homoarginyl, N-Boc homoarginyl, ornithine, N-Boc ornithine, N-benzyl histidyl, lysyl, N-Boc lysyl, N-methyl arginyl, N-methyl guanidine, guanidine and pyridoxyl. L_2 is a linker represented by NH, glycine, N-butyldiamine or guanidine. Examples of this compound is Boc arginine cholesteryl amide (DS46596), N-guanyl-cholesterylamide (DS57511).

In one embodiment the invention features a cationic lipid having the formula IX:



Wherein R is independently a C12-C22 alkyl chain which are saturated or unsaturated, wherein the unsaturation is represented by 1-4 double bonds and R_1 is represented by TREN, N,N'-di-carboxamidine TREN, lysyl, arginyl, ornithyl, homoarginyl, histidyl, aminopropylimidazole, spermine carboxylic acid.

In one embodiment, the invention features process for the synthesis of the compounds of formula I-IX and/or Lipid ID Nos: 700, 701, 705, 709, 719, 732, 736, 737, 738, 739, 742, 743, 744, 751, 752, or 753.

In another embodiment, multi-domain cellular transport vehicles (MCTV) including one or more lipids of formula I-IX and/or Lipid ID Nos: 700, 701, 705, 709, 719, 722, 723, 725, 726, 727, 732, 736, 737, 738, 739, 742, 743, 744, 745, 746, 747, 749, 750, 751, 752, and/or 753, that enhance the cellular uptake and transmembrane permeability of various molecules, for example, nucleic acids, polynucleotides,

oligonucleotides and/or negatively charged molecules in a variety of cell types are provided. Examples of such lipids are shown in Table X. The lipids of the invention are used either alone or in combination with other compounds with a neutral or a negative charge including but not limited to neutral lipid and/or targeting components, to improve the effectiveness of the lipid formulation in delivering and targeting molecules such as nucleic acids, polynucleotides, oligonucleotides, or negatively charged polymers to cells. In addition, these delivery vehicles can be used to increase the transport of other impermeable and/or lipophilic compounds into cells.

In one embodiment, the invention features a compound comprising formula I-IX and/or Lipid ID Nos: 700, 701, 705, 709, 719, 732, 736, 737, 738, 739, 742, 743, 744, 751, 752, or 753. In another embodiment, the invention features a lipid formulation comprising Formulation ID Nos: 282-533.

In another embodiment, the lipid formulation further comprises a targeting component. Targeting components of the invention include ligands for cell surface receptors including, peptides and proteins, glycolipids, lipids, carbohydrates, and their synthetic variants.

In yet another embodiment, the lipid molecules of the invention, such as cationic lipids, are provided as a lipid aggregate, such as a liposome, and co-encapsulated with the compound or polymer to be delivered. Liposomes, which can be unilamellar or multilamellar, can introduce encapsulated material into a cell by different mechanisms. *See*, Ostro, *Scientific American* 102, January 1987. For example, the liposome can directly introduce its encapsulated material into the cell cytoplasm by fusing with the cell membrane. Alternatively, the liposome can be compartmentalized into an acidic vacuole (*i.e.*, an endosome) having a pH below 7.0. This low pH allows ion-pairing of the encapsulated enhancers and the negatively charged polymer, which facilitates diffusion of the enhancer:polymer complex out of the liposome, the acidic vacuole, and into the cellular cytoplasm.

In another embodiment the invention features a lipid aggregate formulation including phosphatidylcholine (of varying chain length; *e.g.*, egg yolk phosphatidylcholine), cholesterol, a cationic lipid, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-polythyleneglycol-2000 (DSPE-PEG_{2000}). The cationic lipid

component of this lipid aggregate can be any cationic lipid known in the art such as dioleoyl 1,2,-diacyl-3-trimethylammonium-propane (DOTAP). In yet another embodiment this lipid aggregate comprises a lipid described in any of the Formulae I-IX and/or Lipid ID Nos: 700, 701, 705, 709, 719, 722, 723, 725, 726, 727, 732, 736, 737, 738, 739, 742, 743, 744, 745, 746, 747, 749, 750, 751, 752, and/or 753,.

In yet another embodiment, polyethylene glycol (PEG) is covalently attached to the lipids of the present invention. The attached PEG can be any molecular weight but is preferrably between 2000-5000 daltons.

The molecules and methods of the present invention are particularly advantageous for introducing nucleic acid molecules into a cell. For example, the invention can be used for nucleic acid delivery, such as enzymatic nucleic acid delivery, where a target site of action exists intracellularly.

In one embodiment, the invention features a lipid formulation, comprising a lipid molecule of the invention or any combination thereof, and a molecule or combination of molecules to be delivered, for example, a nucleic acid, polynucleotide, oligonucleotide, peptide, polypeptide, protein, carbohydrate, steroid, polymer, metal or small molecule.

In another embodiment, the invention features a method of transfecting a cell, comprising contacting a lipid formulation of the invention with the cell under conditions suitable for the transfection.

In one embodiment, a molecule that is complexed with a lipid molecule of the invention includes a nucleic acid, polynucleotide, oligonucleotide, peptide, polypeptide, protein, carbohydrate, steroid, polymer, metal or small molecule.

In one embodiment, a nucleic acid molecule of the invention comprises an enzymatic nucleic acid molecule, antisense nucleic acid molecule, 2-5A antisense chimera, triplex forming oligonucleotide, decoy RNA, dsRNA, siRNA, aptamer, or antisense nucleic acids containing nucleic acid cleaving chemical groups.

A molecule that is complexed with a lipid molecule of the invention includes an enzymatic nucleic acid, for example a hammerhead, Inozyme, G-gleaver, DNAzyme, Amberzyme, Zinzyme and/or allozyme.

In another embodiment, the invention features a method for the delivery of molecules contemplated by the invention, such as nucleic acids, polynucleotides, oligonucleotides, peptides, proteins and/or negatively charged polymers, into cells such as suspension cells and T cells using cytofectins of the present invention.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The drawings will be first described briefly.

Drawings:

Figures 1A-C depict the different classes of lipids of the instant invention.

Figure 2 depicts a scheme for the synthesis of diaminobutyric and guanidinium-based cationic lipids.

Figure 3 depicts a scheme for the synthesis of Boc Arginine cholesteryl amide (12; DS46596).

Figure 4 depicts a scheme for the synthesis of cholesterol-lysine-methyl ester-methylphosphonoamidate (PH55933; 15) and cholesterol-homoarginine-methyl ester methylphosphonoamidate (PH55938; 16).

Figure 5 depicts a scheme for the synthesis of cholesterol-lysine-amide-methylphosphonoamidate (PH55939; 17).

Figure 6 depicts a scheme for the synthesis of cholesterol-TREN-methylphosphonoamidate (PH55941; 18) and cholesterol-TREN-bis-guanidinium methylphosphonoamidate (PH55942; 19).

Figure 7 depicts a scheme for the synthesis of cholesterol-histidine-methylphosphonoamidate (PH55943; 20).

Figure 8 depicts a scheme for the synthesis of cholesterol-aminopropylimidazole-methylphosphonoamidate (PH55945; 21).

Figure 9 depicts a scheme for the synthesis of vitamin-B6 and beta-alanine-based cationic lipids.

Figure 10 depicts a scheme for the synthesis of 2'-aminouridine-based cationic lipids.

Figures 11 depicts a scheme for the synthesis of vitamin-B6-cholesterol conjugate.

Figures 12A-G shows the secondary structure model for seven different classes of enzymatic nucleic acid molecules. Arrow indicates the site of cleavage. --------- indicate the target sequence. Lines interspersed with dots are meant to indicate tertiary interactions. - is meant to indicate base-paired interaction. Figure 12A shows a **Group I Intron motif**: P1-P9.0 represent various stem-loop structures (Cech *et al.,* 1994, *Nature Struc. Bio.*, 1, 273). Figure 12B shows an **RNase P (M1RNA) motif**: EGS represents external guide sequence (Forster *et al.,* 1990, *Science*, 249, 783; Pace *et al.,* 1990, *J. Biol. Chem.,* 265, 3587). Figure 12C shows a **Group II Intron motif:** 5'SS means 5' splice site; 3'SS means 3'-splice site; IBS means intron binding site; EBS means exon binding site (Pyle *et al.,* 1994, *Biochemistry*, 33, 2716). Figure 12D shows a **VS RNA motif:** I-VI are meant to indicate six stem-loop structures; shaded regions are meant to indicate tertiary interaction (Collins, International PCT Publication No. WO 96/19577). Fiogure 12E shows a **HDV Ribozyme motif: :** I-IV are meant to indicate four stem-loop structures (Been *et al.,* US Patent No. 5,625,047). Figure 12F shows a **Hammerhead Ribozyme motif: :** I-III are meant to indicate three stem-loop structures; stems I-III can be of any length and may be symmetrical or asymmetrical (Usman *et al.,* 1996, *Curr. Op. Struct. Bio.,* 1, 527). Figure 12G shows a **Hairpin Ribozyme motif:** H1-H4 are meant to indicate helices 1-4; Helix 1 and 4 can be of any length; Helix 2 is between 3 and 8 base-pairs long; Y is a pyrimidine; B is guanosine, cytidine or uridine; V is adenosine, guanosine, or cytidine (Burke *et al.,* 1996, *Nucleic Acids & Mol. Biol.,* 10, 129; Chowrira *et al.,* US Patent No. 5,631,359).

Figure 13 depicts the structure of fluorescein-conjugated ribozyme and its substrate mRNA sequence. The fluorescein moiety (Fluor), attached through an amino linker, does not reduce the enzymatic activity of the ribozyme.

Figure 14 depicts the chemical synthesis of N^2,N^3-di-oleyl-(N,N'-diguanidinoethyl-aminoethane)-2,3-diaminopropionic acid.

Figure 15 depicts the results of a blood clearance study using the EPC:CHOL:DOTAP:$DSPE_{2000}$ liposome.

Figure 16 depicts the results of a cellular inhibition study of IMPDH-2 mRNA expression in Jurkat cells treated for 24 hours with IMPDH antisense and lipid NC 266.

Figure 17 depicts the results of a cellular inhibition study of IMPDH-2 mRNA expression in Jurkat cells treated for 24 hours with IMPDH antisense and lipid NC 267.

Figure 18 depicts the results of a cellular inhibition study of IMPDH-2 mRNA expression in Jurkat cells treated for 24 hours with IMPDH antisense and lipid NC 388.

Cationic lipids are bifunctional reagents (cationic head group conjugated to a lipid tail) that include a positively charged group that can ion-pair with an anionic group present in a negatively charged polymer, such as a phosphate group present in a nucleic acid phosphodiester linkage or a phosphorothioate group present in nucleic acid having a modified phosphodiester linkage. In one embodiment, the cationic group ion-pairs with a negatively charged polymer to form a lipid:polymer complex, such as a complex with a polynucleotide or a polypeptide (*e.g.* RNA, DNA, and protein). In another embodiment, the cationic group ion-pairs with RNA having enzymatic activity, such as a ribozyme. Formation of the ion-pair increases the intrinsic hydrophobicity of the negatively charged polymer and facilitates diffusion of the polymer across a cell membrane into the cell. The lipid:polymer complex can contain more than one lipid molecule. In one embodiment, a lipid:polymer complex contains lipid in an amount to ion-pair with at least 50% of the anionic groups of a negatively charged polymer. In another embodiment, a lipid:polymer complex contains lipid in an amount to ion-pair with at least 90% of the anionic groups of a negatively charged polymer. The lipid of an lipid:polymer complex can be the same or different. For example, the complex can contain lipids differing in the cationic groups. The amount of cationic lipid and negatively charged polymer which are combined to achieve the desired amount of ionic pairing depends on the environment in which the lipid and the polymer is mixed, the type of lipid, and the type of polymer. The degree of ionic pairing can be measured by techniques known in the art (see, for example, U.S. Patent No. 5, 583, 020, the contents of which are incorporated by reference herein). In another embodiment, the lipid

molecule of the invention is provided in an amount of at least two to ten times per negative charge on the polymer molecule.

Cationic lipids represent a subset of compounds in the broader class of multi-domain cellular transport vehicles (MCTVs). The MCTV family of the invention includes single compounds as well as multi-component delivery systems that incorporate structural domains designed to improve membrane permeability, cellular targeting, while reducing the nonspecific interactions and toxicity of the incoming compound. In addition to cationic lipids, examples of MCTVs include transporters such as facial amphiphiles and other amphipathic compounds, carriers with targeting elements such as glycated moieties, peptides and vitamins, and liposomes with fusogenic elements, pegylated lipids, and/or pH-sensitive components.

The term "nucleic acid molecule" as used herein refers to a molecule comprising nucleotides. The nucleic acid can be single, double, or multiple stranded and can comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof. Nucleic acid molecu, for example, oligonucleotides, enzymatic nucleic acid molecules, ribozymes, DNAzymes, antisense oligonucleotides, 2-5 antisence chimera, triplex forming oligonucleotides, aptamers and other molecules described herein, templates, primers, nucleic acid sensor molecules, reporter and or signal molecules.

The term "negatively charged molecules" as used herein, includes molecules such as naturally occurring and chemically modified nucleic acid molecules (*e.g.*, RNA, DNA, oligonucleotides, mixed polymers, peptide nucleic acid, and the like), peptides (*e.g.*, polyaminoacids, polypeptides, proteins and the like), nucleotides, pharmaceutical and biological compositions, that have negatively charged groups that can ion-pair with the positively charged head group of the lipids of the invention.

The term "compounds with neutral charge" as used herein refers to compositions which are neutral or uncharged at neutral or physiological pH. Examples of such compounds include cholesterol (i.e., a steroidal alcohol, as defined in Lehninger, Biochemistry, 1982 ed., Worth Pub., p. 315) and other steroids, cholesteryl hemisuccinate (CHEMS), dioleoyl phosphatidyl choline, distearoylphosphotidyl choline (DSPC), fatty acids such as oleic acid, phosphatidic acid and its derivatives,

phosphatidyl serine, polyethylene glycol -conjugated phosphatidylamine, phosphatidylcholine, phosphatidylethanolamine and related variants, prenylated compounds including farnesol, polyprenols, tocopherol, and their modified forms, diacylsuccinyl glycerols, fusogenic or pore forming peptides, dioleoylphosphotidylethanolamine (DOPE), ceramide and the like.

The term "head group" as used herein refers to an amino-containing moiety that is positively charged and is capable of forming ion pairs with negatively charged regions of biopolymers such as nucleic acid molecules.

The term "lipophilic group" as used herein refers to a hydrophobic lipid-containing group that facilitates transmembrane transport of the cationic lipid.

The term "linker" as used herein refers to a 1-6 atom carbon chain that links the head group with the lipophylic group.

The term "ion pair" as used herein refers to a non-covalent interaction between oppositely charged groups.

The term "alkyl" as used herein refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. In one embodiment, the alkyl group has 1 to 12 carbons. In another embodiment the alkyl is a lower alkyl of from 1 to 7 carbons, for example 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) can be for example a, hydroxy, cyano, alkoxy, NO_2 or $N(CH_3)_2$, amino, or SH group.

The term "alkoxy" as used herein refers to an OR group, wherein R is an alkyl.

The terms "aryl" as used herein refers to an aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which can be optionally substituted. Non-limiting examples of substituent(s) that can exist on aryl groups are halogen, trihalomethyl, hydroxyl, SH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and/or amino groups.

The term "alkenyl" as used herein refers to unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. In one embodiment, the alkenyl group has 1 to 12 carbons. In another embodiment, the alkenyl group is a lower alkenyl of from 1 to 7 carbons, for example 1 to 4 carbons. The alkenyl group can be substituted or unsubstituted. When

substituted the substituted group(s) can include, hydroxyl, cyano, alkoxy, NO_2, halogen, $N(CH_3)_2$, amino, and/or SH groups.

The term "alkynyl" as used herein refers to an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. In one embodiment, the alkynyl group has 1 to 12 carbons. In another embodiment the alkynyl group is a lower alkynyl of from 1 to 7 carbons, for example 1 to 4 carbons. The alkynyl group can be substituted or unsubstituted. When substituted the substituted group(s) can include, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino and/or SH groups.

The term "alkylaryl" as used herein refers to an alkyl group (as described above) covalently joined to an aryl group (as described above).

The terms "carbocyclic aryl" as used herein refers to groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted.

The terms "heterocyclic aryl" as used herein refers to groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted.

The term "acyl" as used herein refers to -C(O)R groups, wherein R is an alkyl or aryl.

The term "lipid aggregate" as used herein refers to a lipid-containing composition (i.e., a composition comprising a lipid according to the invention) wherein the lipid is in the form of a liposome, micelle (non-lamellar phase) or other aggregates with one or more lipids.

The term "lipid formation" as used herein refers to a formulation comprising at least one lipid molecule, such as those described herein, and at least one molecule to be delivered, for example, a nucleic acid, polynucleotide, oligonucleotide, peptide, polypeptide, protein, carbohydrate, steroid, polymer, metal, or small molecule.

The term “suspension cells” as used herein refers to cells that do not require the attachment to a solid substrate (e.g. plastic surface) (anchorage dependence), in order to survive or proliferate. Non-limiting examples of these cells include hemopoietic, transformed, or cancer cells.

The term “T cell” as used herein refers to cells responsible for cell mediated immunity which originate in the thymus gland (e.g. jurkat cells).

The terms “oligonucleotide” or “polynucleotide” as used herein refers to a nucleic acid molecule comprising a stretch of three or more nucleotides.

The term "enzymatic nucleic acid molecule" as used herein refers to a nucleic acid molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave target RNA. That is, the enzymatic nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. These complementary regions allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA and thus permit cleavage. One hundred percent complementarity is preferred, but complementarity as low as 50-75% can also be useful in this invention (see for example Werner and Uhlenbeck, 1995, Nucleic Acids Research, 23, 2092-2096; Hammann et al., 1999, Antisense and Nucleic Acid Drug Dev., 9, 25-31). The nucleic acids can be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, aptazyme or aptamer-binding ribozyme, regulatable ribozyme, catalytic oligonucleotides, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All of these terminologies describe nucleic acid molecules with enzymatic activity. The specific enzymatic nucleic acid molecules described in the instant application are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving and/or ligation activity to the molecule (Cech et al., U.S. Patent No. 4,987,071; Cech et al., 1988, 260 JAMA 3030).

Several varieties of enzymatic nucleic acids are known presently, which can catalyze, for example, the hydrolysis of RNA phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions. In general, enzymatic nucleic acids with RNA endonuclease activity act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid, for example, first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the enzymatic nucleic acid is a highly specific inhibitor of gene expression, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of an enzymatic nucleic acid molecule.

The term "sufficient length" as used herein refers to an oligonucleotide of greater than or equal to 3 nucleotides that is of a length great enough to provide the intended function under the expected condition. For example, for binding arms of enzymatic nucleic acid "sufficient length" means that the binding arm sequence is long enough to provide stable binding to a target site under the expected binding conditions. Preferably, the binding arms are not so long as to prevent useful turnover of the nucleic acid molecule.

The term "stably interact" as used herein refers to interaction of the oligonucleotides of the invention with a target nucleic acid (e.g., by forming hydrogen bonds with complementary nucleotides in the target under physiological conditions) that is sufficient for an intended purpose (e.g., cleavage of target RNA by an enzyme).

By "antisense nucleic acid", as used herein refers to a non-enzymatic nucleic acid molecule that binds to target nucleic acid by means of RNA-RNA or RNA-DNA or

RNA-PNA (protein nucleic acid; Egholm *et al.,* 1993 *Nature* 365, 566) interactions and alters the activity of the target nucleic acid (for a review, see Stein and Cheng, 1993 *Science* 261, 1004 and Woolf *et al.,* US patent No. 5,849,902). Typically, antisense molecules are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule can bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule can bind such that the antisense molecule forms a loop. Thus, the antisense molecule can be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule can be complementary to a target sequence or both. For a review of current antisense strategies, see Schmajuk *et al.,* 1999, *J. Biol. Chem.,* 274, 21783-21789, Delihas *et al.,* 1997, *Nature,* 15, 751-753, Stein *et al.,* 1997, *Antisense N. A. Drug Dev.,* 7, 151, Crooke, 2000, *Methods Enzymol.,* 313, 3-45; Crooke, 1998, *Biotech. Genet. Eng. Rev.,* 15, 121-157, Crooke, 1997, *Ad. Pharmacol.,* 40, 1-49. In addition, antisense DNA can be used to target nucleic acid by means of DNA-RNA interactions, thereby activating RNase H, which digests the target nucleic acid in the duplex. The antisense oligonucleotides can comprise one or more RNAse H activating region, which is capable of activating RNAse H cleavage of a target nucleic acid. Antisense DNA can be synthesized chemically or expressed via the use of a single stranded DNA expression vector or equivalent thereof.

By "RNase H activating region" as used herein refers to a region (generally greater than or equal to 4-25 nucleotides in length, preferably from 5-11 nucleotides in length) of a nucleic acid molecule capable of binding to a target nucleic acid to form a non-covalent complex that is recognized by cellular RNase H enzyme (see for example Arrow *et al.,* US 5,849,902; Arrow *et al.,* US 5,989,912). The RNase H enzyme binds to the nucleic acid molecule-target nucleic acid complex and cleaves the target nucleic acid sequence. The RNase H activating region comprises, for example, phosphodiester, phosphorothioate (preferably at least four of the nucleotides are phosphorothiote substitutions; more specifically, 4-11 of the nucleotides are phosphorothiote substitutions); phosphorodithioate, 5'-thiophosphate, or methylphosphonate backbone chemistry or a combination thereof. In addition to one or more backbone chemistries

described above, the RNase H activating region can also comprise a variety of sugar chemistries. For example, the RNase H activating region can comprise deoxyribose, arabino, fluoroarabino or a combination thereof, nucleotide sugar chemistry. Those skilled in the art will recognize that the foregoing are non-limiting examples and that any combination of phosphate, sugar and base chemistry of a nucleic acid that supports the activity of RNase H enzyme is within the scope of the definition of the RNase H activating region and the instant invention.

By "2-5A antisense chimera" as used herein refers to an antisense oligonucleotide containing a 5'-phosphorylated 2'-5'-linked adenylate residue. These chimeras bind to target nucleic acid in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which, in turn, cleaves the target nucleic acid (Torrence *et al.*, 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300; Silverman *et al.*, 2000, *Methods Enzymol.*, 313, 522-533; Player and Torrence, 1998, *Pharmacol. Ther.*, 78, 55-113).

By "triplex forming oligonucleotides" as used herein refers to an oligonucleotide that can bind to a double-stranded polynucleotide, such as DNA, in a sequence-specific manner to form a triple-strand helix. Formation of such triple helix structure has been shown to inhibit transcription of the targeted gene (Duval-Valentin *et al.*, 1992 *Proc. Natl. Acad. Sci. USA* 89, 504; Fox, 2000, *Curr. Med. Chem.*, 7, 17-37; Praseuth *et. al.*, 2000, *Biochim. Biophys. Acta,* 1489, 181-206).

By "nucleic acid decoy molecule", or “decoy” as used herein refers to a nucleic acid molecule that mimics the natural binding domain for a ligand. The decoy therefore competes with the natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger et al., 1990, *Cell*, 63, 601-608).

By “aptamer” or “nucleic acid aptamer” as used herein refers to a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that is distinct from sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The

target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein, see for example Gold *et al.,* US 5,475,096 and 5,270,163; Gold *et al.,* 1995, *Annu. Rev. Biochem.,* 64, 763; Brody and Gold, 2000, *J. Biotechnol.,* 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.,* 2, 100; Kusser, 2000, *J. Biotechnol.,* 74, 27; Hermann and Patel, 2000, *Science,* 287, 820; and Jayasena, 1999, *Clinical Chemistry,* 45, 1628.

The term "double stranded RNA" or "dsRNA" as used herein refers to a double stranded RNA molecule capable of RNA interference "RNAi", including short interfering RNA "siRNA" see for example Bass, 2001, *Nature*, 411, 428-429; Elbashir et al., 2001, *Nature*, 411, 494-498; and Kreutzer *et al.,* International PCT Publication No. WO 00/44895; Zernicka-Goetz *et al.,* International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck *et al.,* International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.,* International PCT Publication No. WO 00/44914.

The term "nucleotide" as used herein refers to a heterocyclic nitrogenous base in N-glycosidic linkage with a phosphorylated sugar. Nucleotides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see for example, Usman and McSwiggen, *supra*; Eckstein *et al.,* International PCT Publication No. WO 92/07065; Usman *et al.,* International PCT Publication No. WO 93/15187; Uhlman & Peyman, *supra* all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.,* 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,4,6-trimethoxy

benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*).

By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases can be used at any position, for example, within the catalytic core or substrate binding regions of the enzymatic nucleic acid domain of an nucleic acid sensor molecule. Such modified bases can also be present at one or more positions within the sensor domain of the nucleic acid sensor molecule, for example to improve interaction with the target nucleic acid sequence.

The term "unmodified nucleotide" as used herein refers to a nucleotide with one of the bases adenine, cytosine, guanine, thymine, uracil joined to the 1' carbon of beta-D-ribo-furanose.

The term "modified nucleotide" as used herein refers to a nucleotide that contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate.

By "Inozyme" or "NCH" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described as NCH Rz in Ludwig *et al.*, International PCT Publication No. WO 98/58058 and US Patent Application Serial No. 08/878,640. Inozymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCH/, where N is a nucleotide, C is cytidine and H is adenosine, uridine

or cytidine, and / represents the cleavage site. Inozymes can also possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCN/, where N is a nucleotide, C is cytidine, and / represents the cleavage site

By "G-cleaver" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Eckstein *et al.,* US 6,127,173 and in Kore *et al.*, 1998, *Nucleic Acids Research* 26, 4116-4120. G-cleavers possess endonuclease activity to cleave RNA substrates having a cleavage triplet NYN/, where N is a nucleotide, Y is uridine or cytidine and / represents the cleavage site. G-cleavers can be chemically modified.

By "amberzyme" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Beigelman *et al.,* International PCT publication No. WO 99/55857 and US Patent Application Serial No. 09/476,387. Amberzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NG/N, where N is a nucleotide, G is guanosine, and / represents the cleavage site. Amberzymes can be chemically modified to increase nuclease stability. In addition, differing nucleoside and/or non-nucleoside linkers can be used to substitute the 5'-gaaa-3' loops of the motif. Amberzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

By "zinzyme" motif or configuration is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in Beigelman *et al.,* International PCT publication No. WO 99/55857 and US Patent Application Serial No. 09/918,728. Zinzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet including but not limited to, YG/Y, where Y is uridine or cytidine, and G is guanosine and / represents the cleavage site. Zinzymes can be chemically modified to increase nuclease stability through various substitutions, including substituting 2'-O-methyl guanosine nucleotides for guanosine nucleotides. In addition, differing nucleotide and/or non-nucleotide linkers can be used to substitute the 5'-gaaa-2' loop of the motif. Zinzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

The term “DNAzyme” as used herein refers to an enzymatic nucleic acid molecule that does not require the presence of a 2’-OH group within it for its activity. In particular embodiments the enzymatic nucleic acid molecule can have an attached linker(s) or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2’-OH groups. DNAzymes can be synthesized chemically or expressed endogenously *in vivo,* by means of a single stranded DNA vector or equivalent thereof. Examples of DNAzymes are generally reviewed in Usman *et al.,* International PCT Publication No. WO 95/11304; Chartrand *et al.,* 1995, *NAR* 23, 4092; Breaker *et al.,* 1995, *Chem. Bio.* 2, 655; Santoro *et al.,* 1997, *PNAS* 94, 4262; Breaker, 1999, *Nature Biotechnology,* 17, 422-423; and Santoro *et. al.,* 2000, *J. Am. Chem. Soc.,* 122, 2433-39; Perrin *et al.,* 2001, *JACS.,* 123, 1556. Additional DNAzyme motifs can be selected for using techniques similar to those described in these references, and hence, are within the scope of the present invention.

The term “RNA” as used herein refers to a molecule comprising at least one ribonucleotide residue. By “ribonucleotide” or “2’-OH” is meant a nucleotide with a hydroxyl group at the 2’ position of a β-D-ribo-furanose moiety.

The term “system” or “biological system” as used herein refers to a group of substances or components that can be collectively combined or identified. A system can comprise a biological system, for example, an organism, cell, or components, extracts, and samples thereof. A system can further comprise an experimental or artificial system, where various substances or components are intentionally combined together. The “biological system” as used herein can be a eukaryotic system or a prokaryotic system, for example, a bacterial cell, plant cell or a mammalian cell, or of plant origin, mammalian origin, yeast origin, Drosophila origin, or archebacterial origin.

The term "cation" as used herein refers to a positively charged molecule.

The term "vitamin" as used herein refers to a small molecule, such as riboflavin, nicotinamide, biotin, thiamine, lipoic acid, retinal, pyridoxal, folate, pantothenic acid, cyanocobalamin, aminopterin, and their respective analogs, which bind to a specific protein and participate directly in enzyme catalysis.

Method of Use

The cationic lipid molecules of the instant invention can be used to administer negatively charged polymers which act as pharmaceutical agents. Pharmaceutical agents prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a patient.

The term "patient" as used herein refers to an organism which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which the compounds of the invention can be administered. Preferably, a patient is a mammal, *e.g.,* a human, primate or a rodent.

Generally, these molecules are used in solution with the negatively charged polymer to be administered (*e.g.*, RNA, DNA or protein) and introduced by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention may also be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions; suspensions for injectable administration; and the like.

The present invention also includes pharmaceutically acceptable formulations of the compounds described above, preferably in combination with the negatively charged polymer to be delivered. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.,* systemic administration, into a cell or patient, preferably a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation to reach a target cell (*i.e.*, a cell to which the negatively charged polymer is desired to be delivered to). For example, pharmacological compositions injected into the blood stream should be soluble. Other

factors are known in the art, and include considerations such as toxicity and forms which prevent the composition or formulation from exerting its effect.

The term "systemic administration" as used herein refers to *in* vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes expose the desired negatively charged polymers, *e.g.*, nucleic acids, to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation which can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach may provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as the cancer cells.

In one embodiment, the invention features the use of the cationic lipids of the invention in a composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer an method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al.* *Chem. Rev.* 1995, **95**, 2601-2627; Ishiwata*et al.*, *Chem. Pharm. Bull.* 1995, **43**, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al.*, *Science* 1995, **267**, 1275-1276; Oku *et al.*, 1995, *Biochim. Biophys. Acta*, **1238**, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al.*, *J. Biol.*

Chem. 1995, **42**, 24864-24870; Choi *et al.,* International PCT Publication No. WO 96/10391; Ansell *et al.,* International PCT Publication No. WO 96/10390; Holland *et al.,* International PCT Publication No. WO 96/10392; all of these are incorporated by reference herein). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen. All of these references are incorporated by reference herein.

The present invention also includes compositions prepared for storage or administration which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985) hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents may be provided. *Id.* at 1449. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents may be used. *Id.*

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical

formulation comprising a molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum

acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The molecules of the invention can also be administered in the form of suppositories, e.g., for rectal administration of a compound. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

Molecules of the invention can be administered parenterally in a sterile medium. A drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per patient per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

It is understood that the specific dose level for any particular patient depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The molecules of the present invention can also be administered to a patient in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

The examples provided herein illustrate different aspects and embodiments of the present invention. Although the examples presented here primarily pertain to delivery of ribozymes and plasmid DNA, one skilled in the art will recognize that any nucleic acid, protein, lipid, or another molecule, either alone or in combinations can be delivered to target biological system using the teachings of the present invention. These examples are not intended in any way to limit the disclosed invention.

Example 1: Synthesis of diaminobutyric acid and guanidinium-based cationic lipids (Figure 2)

Synthesis of palmityloleylamine (1): 1-bromohexadecane (15.27 g, 50 mmol) was rapidly added to oleylamine (26.75 g, 100 mmol) at 100 °C. The reaction mixture was heated at 120 °C for 30 minutes and than cooled to room temperature. Chloroform(200 ml) was added followed by 1 N NaOH (50 ml). The mixture was then extracted with H_2O (200 ml), the organic layer dried (Na_2SO_4) and concentrated to a syrup. Silica gel column chromatography using 5-20% gradient methanol in dichloromethane afforded 20.5 g of palmityloleylamine as a syrup (yield, 83%). The identity of the product was confirmed using NMR spectroscopy. ^1H NMR ($CDCl_3$) d 5.34 (m, 2H, CH=CH), 2.58

(m, 4H), 2.00 (m, 4H), 1.47 (m, 4H), 1.25 (m, 48H), 0.86 (m, 6H). FAB-MS: 493 $[M+H]^+$. (Other reagents could include oleyl-bromide and hexadecane amine)

Synthesis of N'-palmityl-*N'*-oleyl-*N*-CBZ-glycinamide (2): (**1**) (2.46 g, 5 mmol) was added to a solution of *N*-CBZ-glycine *N*-hydroxysuccinimide ester (3.06 g, 10 mmol) suspended in dichloromethane (1.39 ml) containing triethylamine (TEA)(10 mmol). The reaction mixture was stirred at room temperature overnight and then concentrated to an oil under vacuum. Silica gel chromatography using 1-5% gradient methanol in dichloromethane gave 1.54 g of *N'*-palmityl-*N'*-oleyl-*N*-CBZ-glycinamide (yield, 45%). ^{1}H NMR ($CDCl_3$) d 7.35 (m, phenyl), 5.83 (br s, NH), 5.35 (m, CH=CH), 5.12 (s, 2H, CH_2Ph), 4.00 (m, 2H, glycyl), 3.31 (m, 2H), 3.13 (m, 2H), 2.00 (m, 4H), 1.53 (m, 4H), 1.25 (m, 48H), 0.88 (m, 6H).

Synthesis of N'-palmityl-*N'*-oleyl-glycinamide (**3**): 10% Palladium on Carbon (Pd/C) was added to *N'*-palmityl-*N'*-oleyl-*N*-CBZ-glycinamide (0.5 g, 0.73 mmol) dissolved in absolute ethanol (3 ml) under argon gas. The flask was immersed in a 20 ^{0}C water bath prior to the addition of 1,4-cyclohexadiene (0.66 ml). The reaction mixture was stirred at room temperature overnight, the catalyst was filtered off and the filtrate evaporated to dryness giving 0.3 g of product (yield, 75%). ^{1}H NMR (acetone-d_6) d 5.41 (m, 2H, CH=CH), 4.07 (br s, 2H, glycyl), 3.36 (m, 2H), 3.29 (m, 2H), 2.80 (br s, NH_2), 2.05 (m, 2H), 1.98 (m, 2H), 1.63 (m, 2H), 1.25 (m, 48H), 0.87 (m, 6H). FAB-MS: 549 $[M+H]^+$.

Synthesis of N'-palmityl-N'-oleyl-alpha,gamma-bis-Boc-diaminobutyryl-glycinamide (4): The mixture of **3** (1.12 g, 2.04 mmol), *N*-alpha-*N*-gamma-di-Boc-diaminobutyric acid (631 mg, 2.24 mmol), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) (553 mg, 2.24 mmol) in CH_2Cl_2 was stirred for 1 hour at room temperature. The mixture was then concentrated to a syrup and 1.2 g of the product was isolated by column chromatography using 20-50% gradient of hexanes in ethyl acetate (yield, 69%). ^{1}H NMR ($CDCl_3$) d 7.14 (br s, NH), 5.38 (m, 2H, CH=CH), 5.28 (br s, 1H, NH), 5.12 (br s, 1H, NH), 4.02 (m, 2H, glycyl), 3.42 (m, 1H), 3.31 (m,

2H), 3.15 (m, 2H), 3.02 (m, 2H), 1.95 (m, 4H), 1.77 (m, 2H), 1.53 (m, 4H), 1.25 (m, 48 H). FAB-MS: 850 $[M+H]^+$.

***Synthesis of N'-palmityl-N'-oleyl-alpha, gamma-diaminobutyryl-glycinamide (JA59311)(5)*:** compound **4** (350 mg, 0.41 mmol) was dissolved in dioxane (6 ml) followed by the addition of 4 M HCl in dioxane (6 ml). The reaction mixture was stirred at room temperature for 2 hours, than concentrated *in vacuo* and azeotroped twice with toluene. The residue was partitioned between CH_2Cl_2 and 0.2 N NaOH, the organic layer was washed with saturated $NaHCO_3$ solution, and then dried (Na_2SO_4) and evaporated to dryness. Flash silica gel chromatography using CH_2Cl_2/methanol/conc. NH_4OH 40:10:2 yielded 200 mg of compound **5** (yield, 75%). ^1H NMR ($CDCl_3$) d 8.06 (br s, 1H, NH), 5.38 (m, 2H, CH=CH), 4.04 (m, 2H, glycyl), 3.54 (m, 1H), 3.31 (m, 2H), 3.17 (m, 2H), 2.86 (m, 2H), 1.93 (m, 4H), 1.67 (m, 2H), 1.54 (m, 4H), 1.41 (br s, 4H, NH_2), 1.25 (m, 48H), 0.87 (m, 6H). FAB-MS: 650 $[M+H]^+$.

***Synthesis of N'-palmityl-N'-oleyl-N-gamma-carboxamidine-alpha,gamma-diaminobutyryl-glycinamide* (JA59312) (6):** To the solution of **5** (0.16 g, 0.25 mmol) and diisopropylethylamine (DIPEA) (83 mL) in THF/methanol 1:1 (0.8 ml), 1*H*-pyrazole-1-carboxamidine hydrochloride (70 mg, 0.48 mmol) was added under argon gas. The reaction mixture was stirred at room temperature overnight and then concentrated to a syrup. Silica gel column chromatography using CH_2Cl_2 followed by CH_2Cl_2/methanol/conc. NH_4OH 40:10:2 yielded 50 mg of compound **6** (yield, 29%). ^1H NMR ($CDCl_3$) d 5.37 (m, 2H, CH=CH), 4.07 (m, 2H, glycyl), 3.94 (m, 1H), 3.42 (m, 2H), 3.21 (m, 4H), 2.01 (m, 6H), 1.58 (m, 4H), 1.46 (m, 2H, NH_2), 1.25 (m, 48H), 0.87 (m, 6H). FAB-MS: 692 $[M+H]^+$.

Synthesis of N'-palmityl-N'-oleyl-alpha,gamma-bis-trimethylammoniumbutyryl-glycinamide (JA59316)(7): A dihydrogenchloride salt of **5** (130 mg, 0.2 mmol) was dissolved in methanol (4 ml) and combined with $KHCO_3$ (0.2 g) and CH_3I (0.2 ml). The mixture was then stirred at room temperature for 3 days. The

reaction mixture was then filtered through the bed of Celite, followed by filtration through 0.45m PTFE filter. The filtrate was then evaporated to dryness affording 160 mg of the desired product (yield, 94%). ^{1}H NMR ($CDCl_3$) d 3.58 [s, 9H, $(CH_3)_3$], 3.44 [s, 9H, $(CH_3)_3$].

Synthesis of N'-palmityl-N'-oleyl-N-carboxamidine-glycinamide•HCl (JA59314)(8): Using the same procedure described above for the preparation of **6** except that crystallization from methanol instead of column chromatography was used for purification, **8** was prepared in 51% yield. ^{1}H NMR ($CDCl_3$) d 7.70-7.25 (m, 5H, NH), 5.38 (m, 2H, CH=CH), 4.25 (m, 2H, glycyl), 3.27 (m, 4H), 1.96 (m, 4H), 1.53 (m, 4H), 1.25 (m, 48 H), 0.87 (m, 6H). FAB-MS: 592 $[M+H]^+$.

Synthesis of N'-palmityl-N'-oleyl-guanidine (JA59317) (9): The mixture of a hydrochloride salt of **1** (285 mg, 0.54 mmol) , cyanamide (50 mg, 1.19 mmol) and 1-butanol (2 ml) was stirred at 120 ^{o}C for 2 hours. The cooled mixture was diluted with CH_2Cl_2(50 ml) and washed with saturated aqueous NaCl(Brine)/methanol 1:1 (50 ml). The organic layer was then dried (Na_2SO_4), evaporated to an oil and chromatographed on a column of silica gel using CH_2Cl_2/methanol/conc. NH_4OH 40:10:2 giving 80 mg of the desired material (yield, 28%). ^{1}H NMR ($CDCl_3$) d 7.08 (br s, 1H, NH), 5.34 (m, 2H, CH=CH), 3.29 (m, 4H), 2.00 (m, 4H), 1.62 (m, 4H), 1.25 (m, 48H), 0.88 (m, 6H). FAB-MS: 535 $[M+H]^+$.

Example 2: Synthesis of DS 46596 (12)

*Synthesis of Cholesterylamine (**10**):* Referring to Figure 3, cholesteryl chloride (10 grams, 25mmol) was partially dissolved in dry methanol (50 ml) and the solution was heated with stirring to 155°C for 18 hr at 500 psig using a 300 ml Parr bomb apparatus charged with dry ammonia gas. The bomb was cooled to room temperature and the methanol was removed by steam distillation on a rotary evaporator. Compound **10** was purified using E. Merck silica chromatography by eluting with dichloromethane / methanol (4:1 v/v) to yield 4 grams of the ninhydrin positive product (yield, 60%). Identity was confirmed by ES-MS.

Synthesis of Boc$_3$arginineNHcholesterylamide (11): A 200 mL pear shaped flask with stir bar was charged with a mixture of **10** (1g, 2.6 mmol) , Boc$_3$ arginine (1.2 grams, 2.6 mmol), diisopropylcarbodiimide (450 ul, 2.9 mmol) and dichloromethane (70 mls). The mixture of reagents was stirred at room temperature for two hours. Following the reaction, the solution was washed with aqueous sodium bicarbonate (5% w/v) and the organic layer was separated and dried to a solid using a rotary evaporator. Compound **11** was dissolved in 5 mls of dichloromethane prior to purification using silica gel chromatography. (yield, 90%). Identity was confirmed by ES-MS. ^{1}H NMR (dmso-d6): . ^{1}H NMR (dmso-d6): 9.32 (bd), 6.63 (d), 5.30 (m), 4.00 (m), 3.80 (m) 3.40 (m), 1.539 (s, tBoc), 1.517 (s, tBoc), 1.497(s, tBoc).

Synthesis of Boc arginineNHcholesterylamide (DS4659) (12): Compound **11** (50 mg, 60 umol) was dissolved in anhydrous 1,4-dioxane (300 ul) and combined with 4M HCl in dioxane (400 ul). The mixture was left at room temperature for 2 hours and the reaction was stopped by removing all solvent and HCl using a stream of dry nitrogen gas. Compound **12** was isolated using a wide pore C18 silica column and an isocratic methanol: water (88:12) eluant with detection at 210 nm. Fractionation allowed recovery of 20 mgs of compound **12** (yield, 44%). Identity was confirmed by ES-MS. ^{1}H NMR (dmso-d6): 7.82 (d), 6.36 (bs), 3.51 (m), 3.43 (m), 3.33 (m) 3.15 (m), 1.472 (s, tBoc).

Example 3: Synthesis of PH 55933 (15) and PH 55938 (16)

Synthesis of (13): Referring to the Figure 4, to a solution of methylphosphonic dichloride (0.332 g, 2.5 mmol, ^{31}P NMR s, 43.93 ppm) stirring at room temperature under positive pressure argon was added 4-dimethylaminopyridine(DMAP) (0.31 g, 2.5 mmol). The resulting clear, colorless solution was cooled to -70 C and a solution of cholesterol (0.97 g, 2.5 mmol) suspended in anhydrous dichloromethane (20 ml) was added via syringe with vigorous stirring over a period of one hour. The reaction mixture was allowed to warm to room temperature and was maintained at room temperature for 18 hours at which time ^{31}P NMR analysis of a small aliquot of the reaction mixture indicated complete reaction (d, 39.08 ppm).

Crude **(13)** was treated with additional DMAP (0.31 g, 2.5 mmol) and the reaction mixture cooled to -70 C while stirring under positive pressure argon. H-Lys(Z)-OCH_3 (0.66 g, 2.25 mmol) in anhydrous dichloromethane (20 ml) was added dropwise via syringe over a period of one hour. The reaction mixture was warmed to room temperature and stirred for an additional 18 hours (reaction complete by ^{31}P NMR). Direct loading onto flash silica followed by a gradient of 0 to 10% EtOAc/hexanes then 5% EtOH/dichloromethane gave 1.12 g of **(14)** (Yield, 60% over two steps). ^{31}P NMR (s, 30.98 ppm).

Synthesis of Cholesterol-Lysine-methylphosphonoamidate (PH55933) (15): Compound **(14)** (1.0g, 1.35 mmol) was dissolved in anhydrous EtOH and cooled to 0 C with an ice/water bath while stirring under argon. 10 Pd/c (1.0 g, 1 mass eq.) was added to the reaction mixture followed by dropwise addition of 1,4 cyclohexadiene (1.27 ml, 13.5 mmol). After warming to room temperature, the reaction was complete after 4 hours as determined by TLC (15% MeOH/dichloromethane). The reaction mixture was filtered over celite and dried *in vacuo*. Flash chromatography utilizing a gradient of 5 to 15% MeOH/dichloromethane 1% TEA afforded 0.64 g of **(15)**: (yield, 78%) ^{31}P NMR (s, 30.88 ppm), mass spec. calcd=606.87, found=607.47.

Synthesis of Cholesterol-Homoarginine-methylphosphonoamidate **(PH55938) (16):** To a solution of **(15)** (0.131 g, 0.216 mmol) stirring at room temperature under argon in anhydrous DMF (2.0 ml), 1-H-pyrazole-1-carboxamidine•HCl (32 mg, 0.216 mmol) was added followed by diisopropylethylamine (28 ml, 0.216 mmol). The reaction mixture was stripped slightly on a rotovap then rotated overnight without vacuum at room temperature. After removing DMF *in vacuo* the reaction residue was dissolved in dichloromethane and applied to a flash silica gel column. An isocratic system of 20% MeOH/dichloromethane, 2% NH_4OH followed by treatment with Dowex OH^- (300 mg) in MeOH gave 80 mg of desired product (yield, 57%). ^{31}P NMR (s, 31.99 ppm), mass spec. calcd=648.91, found=649.48.

Example 4: Synthesis of PH 55939 (17):

*Synthesis of Cholesterol-Lysine-methylphosphonoamidate amide (***PH55939) (17)**: Refering to Figure 5, compound **(15)** (76 mg, 0.125 mmol) was treated with a 0 C saturated methanolic ammonia solution (5 ml) at room temperature for 18 hours (some venting required). The reaction mixture was evaporated *in vacuo* then purified by flash silica gel chromatography to give 45 mg of product (yield,61%). ^{31}P NMR (s, 32.17 ppm), mass spec. calcd=591.86, found=592.23.

Example 5: Synthesis of PH 55941 (18) and PH 55942 (19)

*Synthesis of Cholesterol-TREN-methylphosphonamidate (***PH55941) (18)**: Refering to Figure 6, 4-Dimethylaminopyridine (DMAP) (0.31 g, 2.5 mmol) was added to a solution of methylphosphonic dichloride (0.332 g, 2.5 mmol, ^{31}P NMR s, 43.93 ppm) stirring at room temperature under positive pressure argon gas. The resulting clear, colorless solution was cooled to -70 C and a solution of cholesterol (0.97 g, 2.5 mmol) in anhydrous dichloromethane (20 ml) was added via syringe with vigorous stirring over a period of one hour. The reaction mixture was allowed to warm to room temperature and was maintained for 18 hours at which time ^{31}P NMR analysis of a small aliquot of the reaction mixture indicated complete reaction (d, 39.08 ppm). Crude **(13)** was treated with additional DMAP (0.31 g, 2.5 mmol) and the reaction mixture cooled to -70 C while stirring under positive pressure argon. Tris(2-aminoethyl)amine (TREN) (0.37 ml, 2.5 mmol) in anhydrous dichloromethane (20 ml) was added dropwise via syringe over a period of two hours. The reaction mixture was warmed to room temperature and stirred for an additional 18 hours (reaction complete by ^{31}P NMR). Direct loading onto flash silica followed by a gradient of 10 to 20% MeOH/dichloromethane with 1 to 4% NH_4OH gave 0.442 g of **(18)** as a white foam: (yield,28% over two steps), ^{31}P NMR (d, 32.57 ppm), mass spec. calcd=592.89, found=593.49.

*Synthesis of Cholesterol-TREN-bis-guanidinium methylphosponamidate (***PH55942) (19)**: Compound **(18)** (0.148 g, 0.25 mmol) was dissolved in anhydrous DMF (1.0 ml) and anhydrous dichoromethane (5.0 ml). 1-H-pyrazole-1-

carboxamidine•HCl (73 mg, 0.50 mmol) was added to the reaction mixture followed by diisopropylethylamine (87 ml, 0.50 mmol). Dichloromethane was stripped off of the reaction mixture on a rotovap then rotation continued overnight without vacuum at room temperature. After removing DMF *in vacuo* the reaction residue was dissolved in dichloromethane and applied to a flash silica gel column. A gradient of 5 to 20% MeOH/dichloromethane with 0.5 to 2% NH_4OH followed by treatment with Dowex OH^- (300 mg) in MeOH gave pure **(19)**: 0.11 g, 65%, ^{31}P NMR (d, 33.83 ppm), mass spec. calcd=676.97, found=677.54.

Example 5: Synthesis of PH 55943

*Synthesis of Cholesterol-Lysine-methylphosphonoamidate (***PH55943) (20)**: Refering to Figure 7, crude **(13)** was treated with additional DMAP (0.31 g, 2.5 mmol) and the reaction mixture cooled to -70 C while stirring under positive pressure argon. H-His(Bzl)OCH_3 (0.65 g, 2.5 mmol) in anhydrous dichloromethane (20 ml) was added dropwise via syringe over a period of one hour. The reaction mixture was then warmed to room temperature and stirred for 18 hours (reaction complete by ^{31}P NMR). Direct loading onto flash silica followed by a gradient of 2 to 10% EtOAc/hexanes then 0 to 5% MeOH/dichloromethane gave 0.53 g of **(20)** (30% over two steps), ^{31}P NMR (d, 31.39 ppm), mass spec. calcd=705.96, found=706.47.

Example 6: Synthesis of PH 55945 (21)

*Synthesis of Cholesterol-Histidine-methylphosphonamidate (***PH55945) (21)**: Refering to Figure 8, crude **(13)** was treated with additional DMAP (0.31 g, 2.5 mmol) and the reaction mixture cooled to -70 C while stirring under positive pressure argon. 1-(3-aminopropyl)-imidazole (0.30 ml, 2.5 mmol) in anhydrous dichloromethane (20 ml) was added dropwise via syringe over a period of one hour. The reaction mixture was warmed to rt and stirred at rt for an additional 18 hours (reaction complete by ^{31}P NMR). Direct loading onto flash silica after saturated bicarb washing followed by a gradient of 0 to 10% EtOAc/hexanes then 0 to 10% MeOH/dichloromethane affording 0.77 g of **(21)**: (yield, 54% over two steps), ^{31}P NMR (d, 32.47 ppm), mass spec. calcd=571.82, found=572.33.

Example 7: Synthesis of vitamin B6 and beta-alanine-based cationic lipids

Synthesis of N-CBZ-beta-Alanine N-hydroxysuccinimide ester (22): Refering to Figure 9, the compound was prepared according to Lewis *et al. PNAS* 1996, **93**, 3176-3181 (incorporated by reference herein). Yield 80%. ^{1}H NMR (DMSO-d_6) d 7.42 (t, 1H, NH), 7.33 (m, 5H, benzyl), 5.009 (s, CH_2), 1.47 (m, 4H), 3.32 (m, 2H, CH_2NH), 2.86 (t, 2H,CH_2 CO), 2.79 (s, 4H, CH_2CH_2).

Synthesis of N'-palmityl-N'-oleyl-N-CBZ-beta-alanine amide (23): compound **22** (1.0 g, 2.03 mmol) was added to a solution of *N*-CBZ-beta-Alanine *N*-hydroxysuccinimide ester (0.16 g, 0.5 mmol) in CH_2Cl_2 containing Et_3N (0.42 ml, 3 mmol). The reaction mixture was stirred at room temperature overnight, diluted with dichloromethane and washed with saturated $NaHCO_3$ and brine. Organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using mixture of EtOAc-Hexanes (1:3) as an eluent to give 1.32 g of **23** as a yellow oil(yield, 93%). ^{1}H NMR ($CDCl_3$) d 7.34 (m, phenyl), 5.62 (t, NH), 5.35 (m, CH=CH), 5.082 (s, 2H, *CH_2*Ph), 3.49 (m, 2H, CH_2NH), 3.27 (m, 2H,), 3.15 (m, 2H), 2.5 (m, 2H, CH_2 CO), 2.00 (m, 4H), 1.49 (m, 4H), 1.25 (m, 48H), 0.87 (m, 6H).

Synthesis of N'-palmityl-N'-oleyl-*beta-*alanine amide (AK 524-68) (POABA)(24): compound **23** (1.2 g, 1.72 mmol) was dissolved in absolute ethanol (10 ml) and 10% Pd-C was added under argon. The flask was then immersed in a 20 ^{0}C water bath prior to the addition of 1,4-cyclohexadiene (1.6 ml). The reaction mixture was stirred at room temperature for 40 hours, the catalyst was filtered off and the filtrate evaporated to dryness. The residue was purified by flash chromatography on silica eluting with the linear gradient of MeOH (5% to 10%) in dichloromethane to give 0.68 g, of **24** (yield, 70%). ^{1}H NMR ($CDCl_3$) d 5.37 (m, 2H, CH=CH), 3.26 (m, 4H,), 3.16 (m, 2H), 2.75 (t, NH_2), 1.95 (m, 2H), 1.51 (m, 4H), 1.25 (m, 48H), 0.87 (m, 6H). FAB-MS: 563.6 $[M+H]^+$.

Synthesis of N'-palmityl-N'-oleyl-N-carboxamidine-beta-alanine amide (AK 524-73) (GPPOA) (25): The mixture of **24** (60 mg, 0.11 mmol), pyrazole carboxamidine hydrochloride (16 mg, 0.11 mmol) and diisopropylethylamine (20 mL, 0.12 mmol) in 0.5 mL of THF-MeOH (1:1) was stirred overnight at room temperature. It was then evaporated to dryness, dissolved in dichloromethane and washed with aqueous ammonia. The organic layer was dried over sodium sulfate to give 25 as an yellowish oil. Yield near quantitative. ^{1}H NMR ($CDCl_3$) d 7.70-7.25 (m, 5H, NH), 5.38 (m, 2H, CH=CH), 3.43 (m, 2H, CH_2NH), 3.23 (m, 2H), 3.17 (m, 2H), 2.54 (m 2H, CH_2CO)1.96 (m, 4H), 1.49 (m, 4H), 1.25 (m, 48 H), 0.87 (m, 6H). FAB-MS: 605.6 $[M+H]^+$.

Synthesis of N(N''-palmityl-N''-oleyl-amidopropyl)pyridoxamine (AK 524-74) (POCAEP) (26): Compound 26 was prepared analogously to compound 27 (Yield, 78%). FAB-MS: 714.6 $[M+H]^+$.

Example 8: Synthesis of AK524-76 (27)

N-cholesteryl-pyridoxamine (AK524-76) (CCAEP) (27): Refering to Figure 11, The suspension of Pyridoxal hydrochloride (0.1g, 0.5 mmol) in ethanol was brought to pH 7 with 1N NaOH followed by the addition of aminocholesterol (0.19g, 0.5 mmol). The pH of the resulting bright yellow solution was adjusted to 8 (1N NaOH) and set aside for 10 minutes. Sodium borohydride (20 mg, 0.5 mmol) was then added to the reaction mixture resulting in immediate color disappearance. After 15 minutes reaction mixture was acidified (pH 6) with 1N HCl, diluted with dichloromethane and washed with aqueous ammonia and water. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using 10% MeOH in dichloromethane as an eluent to give 0.25g (93%) of 27 . ^{1}H NMR ($CDCl_3$) d 7.78 (s, 1H, H-6 Pyr), 4.58 (s, 2H, CH_2O), 4.06 (AB-quartet 2H, CH_2N), 2.4 (s, 3H, 2-CH_3), 2.2-0.24 (m, cholesteryl moiety). FAB-MS: 537.4 $[M+H]^+$.

Example 9: Synthesis of 2'-aminouridine-based cationic lipids

2'-Deoxy-2'-(N-Fmoc-beta-alanineamido) uridine (28): Referring to Figure 10, EEDQ (4.2g, 17 mmol) was added to the solution of 2'-amino-2'-deoxy uridine (4 g,

16.45 mmol) and N-FMOC-beta-alanine (5.1 g, 16.45 mmol) in methanol and the reaction mixture was boiled for two hours. Subsequent flash chromatography on silica using a linear gradient of methanol (5% to 10%) in dichloromethane afforded 6 g of 2'-Deoxy-2'-(*N*-Fmoc-beta-alanineamido) uridine (77%). ^{1}H NMR ($CDCl_3$-DMSO-d6) d 10.398 (s, 1H, N3-H), 6.98 - 7.63 (m, H6, Fmoc), 6.16 (t, 1H, NHFmoc), 5.73 (d, 1H, H1', $J_{1',2'}$ 8.4), 5.34 (d, 1H, H5), 4.22 (m, 1H, 2'-H) 3.98 (dd 2H, CH_2), 3.88 (m, 1H, 3'-H), 3.77 (br s, 1H, 4'-H), 3.43 (m, 2H, 5'-CH_2), 3.1 (m, 2H, $C\underline{H}_2$NHFmoc), 2.07 (t, 2H, $C\underline{H}_2$CO).

Synthesis of 3',5'-Di-palmitoyl-2'-deoxy-2'-(N-Fmoc-beta-alanineamido) uridine (29): Palmitoyl chloride (1.55 mL, 1.8 mmol) was added to a solution of nucleoside **28** in abs pyridine and the reaction mixture was stirred overnight at room temperature. The solution was then quenched with MeOH, evaporated to dryness, dissolved in dichloromethane and washed with saturated aq sodium bicarbonate and brine. The organic phase was dried oversodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica (EtOAc-Hexanes 1:1) affording 0.5g of **29** (yield, 65%).

Synthesis of 3',5'-Di-palmitoyl-2'-deoxy-2'-(beta-alanineamido) uridine (AK 524-71) (30): To the solution of **29** (0.5g, 0.49 mmol) in dichloromethane (5 mL) was added morpholine (1 mL) and the reaction mixture was stirred at room temperature for 36 hours. Subsequent flash chromatography on silica using linear gradient of methanol (5% to 10%) in dichloromethane afforded 0.22g of desired product (yield, 56%) of **30** . FAB-MS: 791.6 $[M+H]^+$.

Synthesis of 3',5'-Di-palmitoyl-2'-deoxy-2'-(N-carboxamidine-beta-alanineamido) uridine (AK 524-75) (31): Compound **31** was prepared analogously to compound 2**5.** Yield 80%. FAB-MS: 833.6 $[M+H]^+$.

Example 10: Preparation of lipid-based formulations including cationic lipids and DOPE

For each cationic lipid, four aqueous suspensions were prepared, three containing the fusogenic neutral lipid DOPE (dioleoyl phosphatidyl ethanolamine), and one containing the cationic lipid only (Table II). For this, the solid cationic lipids were

dissolved in chloroform and aliquots transferred to 5 ml glass tubes with Teflon-lined caps. DOPE, also dissolved in chloroform, was then added to individual tubes at 1:1, 2:1, or 3:1 molar ratios (ratio of cationic lipid to DOPE). The lipid mix was deposited as a film in the glass tube by evaporating the solvent with a stream of argon. The lipid film was hydrated with water (1 ml per mg total lipid) and then resuspended by sonication using a bath sonicator (three or four 15 s treatments with intermittent vortex mixing). The formulations were stored at 4 C until used (usually within 8 weeks).

Example 11: Cell culture and synthesis of anionic polymers

Cellular delivery and efficacy assays were carried out in monolayer cultures of cells originating from normal tissues or tumors (Table II, III and V). Cells were maintained in humidified incubators using growth medium recommended by the supplier. Hammerhead ribozymes were synthesized and purified using standard protocols (Wincott *et al.,* 1995, 23, 2677; Beigelman *et al.,* 1995, *J. Biol. Chem.* 270, 25702; both are incorporated by reference herein). Nuclease resistant linkages were incorporated at specific sites of the ribozymes, modifications that markedly increased the serum half-life from a few minutes to several hours. For cellular delivery studies, fluorophore-tagged 32-mer ribozymes were prepared by attaching a fluorescein or rhodamine moiety to the loop portion through a aminolinker-modified base (Figure 13). An expression plasmid encoding the humanized Green Fluorescent Protein (plasmid pEGFP-C1) was obtained from Clontech.

Example 12: Cellular Ribozyme delivery

For the delivery studies, subconfluent cultures of mammalian cells were seeded in 24-well plates (~20,000 cells/well) a day prior to the initiation of assay. In a typical delivery assay, 100 μl of a 1 μM fluorescein or rhodamine-conjugated ribozyme (i.e., 10 X ribozyme diluted in water) was placed in a polystyrene tube, and an aliquot of the cationic lipid formulation was added at room temperature to allow complex formation. The appropriate growth medium added to each tube (0.9 ml) and then the contents were mixed and added to each well. Final concentration of the ribozyme was 100 nM and the transport vehicle concentration was varied from 2.5 to 20 μg/ml. After a 3-4 h incubation, the medium was replaced with normal growth medium and the localization

of the cell-associated ribozyme was evaluated by fluorescence microscopy using a Nikon stage microscope equipped with a 40 X objective and a ccd camera. In some studies, the total cell-associated ribozyme was quantified by FACS analysis.

Example 13: Cellular Plasmid delivery

Subconfluent cultures of cells were seeded in 24-well plates (~10,000 cells/well). In typical transfection studies, 100 ng of the plasmid in 0.1 ml water was premixed with individual lipids in a polyethylene tube and incubated at room temperature for ~10 minutes to allow complex formation. Then 0.9 ml growth medium (serum-free) was added to each tube, the contents were mixed, and administered to individual wells of cells for 3-4 h. The medium was then replaced with normal growth medium and cells were left for ~24 h. Expression of GFP was monitored by fluorescence microscopy. The transport vehicles that led to GFP expression in the highest percentage of cells were identified for each cell line (Table IV).

Example 14: Cytotoxicity analysis

The toxic effects of the lipid-formulated compositions on cells were determined in three ways. First, cellular morphology was evaluated in relation to normal, untreated cells and significant abnormalities or reduction in cell numbers were noted. Second, for evaluating gross toxicity, propidium iodide was added to the medium and the cells were examined for the presence of pink-red fluorescence in the nucleus, indicating the presence of perforated or damaged membranes. Finally, the longer term effect of the treatment on cells was quantified using a sensitive MTS proliferation assay (Promega).

Example 15: c-*myb* proliferation assay

The protooncogene c-*myb* is a transcription factor that participates in regulating the proliferation of smooth muscle cells. It has been demonstrated that cells in which c-*myb* levels have been reduced by ribozyme-Lipofectamine treatment do not proliferate well in response to subsequent serum stimulation. Two ribozymes directed against c-*myb* termed "active" and "inactive" (Jarvis *et al.,* 1995, *RNA*, 2, 419). Both ribozymes can recognize the mRNA target but only the "active" can cleave it. The "inactive" ribozyme serves as a negative control. In principle, the active ribozyme can reduce c-*myb* expression by catalyzing the sequence-specific cleavage of the mRNA, leading to a

reduction in cell proliferation. This assay was used to validate the utility of delivery formulations.3H-Thymidine Incorporation Assay

In typical cell proliferation, subconfluent cultures of rat aortic smooth muscle cells (RASMC) were plated in 48-well plates in DMEM supplemented with amino acids, Hepes, and 10% fetal bovine serum (5000 cells/ well/ 0.5 ml medium). Next day, cells were serum-starved, to inhibit proliferation, by replacing the medium with low serum medium (0.5% serum) for ~2 days. The starved cells were then treated with ribozyme-carrier formulations, usually 100 nM ribozyme premixed with 2.5-10 μg/ml carrier lipid, in serum-free medium for ~2 h, followed by "trafficking" in low serum-medium (0.25% serum) for ~20 h. Triplicate set of wells were exposed to each treatment. The cells were then stimulated to proliferate for 12 h in medium containing 10% serum. This was followed by another 8h incubation in medium+10% serum+ ^{3}H-thymidine (~1 μCi/ml). The cells were then fixed with ice-cold 10% trichloroacetic acid, washed twice with water, and ^{3}H-thymidine incorporated into new DNA was measured by scintillation counting. The inhibition of proliferation using different ribozyme formulations is shown in Table V.

Example 16: Cellular transport of lipophilic compounds

Rhodamine-conjugated dioleoyl phosphatidyl ethanolamine (DOPE) was mixed with various cationic lipids and administered to cells seeded in 24-well plates. After ~3 h incubation, the cellular distribution of the fluorescent rhodamine-DOPE was examined by fluorescence microscopy. Every cell contained rhodamine (red fluorescence), indicating that the lipids were delivered efficiently to the cells. Next, fluorescein-conjugated ribozymes were packaged and coadministered to cells using the same vehicles using procedures described earlier. Again, every cell was labeled with rhodamine while a subset contained internalized ribozymes (green fluorescence). These observations suggested the lipid transporters can be used to deliver lipophilic as well as hydrophilic compounds to cells.

Example 17: Delivery of antisense molecules

A 21-nucleotide long phosphorothioate oligodeoxynucleotide with an attached fluorescein moiety was synthesized by standard procedures. The oligonucleotide (100

nM) was formulated with different concentrations (2.5 to 10 μg/ml) of each transport vehicle and administered to cells. Subcellular distribution of the internalized material was evaluated by fluorescence microscopy. The results indicated that optimal transporter concentrations are different for antisense oligonucleotides compared to ribozymes.

Example 18: Synthesis of N^2,N^3-di-oleyl-(*N,N*'-diguanidinoethyl-aminoethane)-2,3-diaminopropionic acid (**36**)

Referring to Figure 14, applicant describes a reaction of 2,3-diaminopropionic acid **32** with oleoyl chloride in the presence of dimethylaminopyridine (DMAP) and triethylamine (TEA) can give the peracylated derivative **33,** oleyl. Reaction of **33** with triethylenetetramine (TREN) **34,** followed by reaction with 1*H*-Pyrazole-1-carboxamidine hydrochloride **35** can give the title compound **36.**

Example 19: Preparation of PC:CHOL:DOTAP:$DSPE_{2000}$ liposome formulation

Formation of EPC:CHOL:DOTAP:DSPE-PEG$_{2000}$: Egg yolk phosphatidylcholine (EPC), cholesterol, and DOTAP were purchased from Avanti Polar Lipids. DSPE-PEG $_{2000}$ (1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-polythylene glycol-2000) was purchased from Shearwater polymers. Extruder was purchased from Lipex biomembranes. FPLC was purchased from Pharmacia. Radioactive compounds were purchased from NEN and ether from Sigma.

The following lipids suspended in chloroform were mixed together in a 50 ml round bottom flask : phosphatidylcholine (egg yolk) (85.5 mg), cholesterol (21.8 mg), DOTAP (23.7 mg), ceramide-PEG C20 (61.8 mg) resulting in a molar ratio of 50:25:15:10. A tracer of Cholesteryl hexadecyl ether (CHE) (26 μCi) was added to monitor lipid concentration. The lipids were dried down by rotary evaporation and then resuspended in ether (9 ml). Ribozyme (25 mg) suspended in 1X phosphate buffered saline (3 ml) was added to the ether/lipid mixture and mixed together into an emulsion. The ribozyme was quantitated using a ^{32}P internally labeled ribozyme tracer (160 μCi). Liposome vesicles were formed by removing the ether under vacuum. Residual ether was removed by bubbling argon gas through the lipid-ribozyme mixture for 10 minutes.

Vesicles were then passed through polycarbonate filters with 200 nm and 100 nm pores consecutively 6-10 times using an Extruder (Lipex Biomembranes, Vancouver, B. C.). Liposomes were purified from unencapsulated material using an FPLC column packed with DEAE sepharose CL-6B. Ribozyme and lipid concentration was determined by reading an aliquot of purified liposomes on a scintillation counter for both tritium and 32P. The counts indicated that 5.75 mg of ribozyme was present within liposome vesicles (23% encapsulation).

Example 20: Blood clearance study using the EPC:CHOL:DOTAP:$DSPE_{2000}$ liposome

Female C57B1/6J weighing 20-25 g were used in this study. with 3 mmol of lipid (36 mg ribozyme) by tail vein injection. The time points observed were 15 minutes, 1 hour, 4 hour, and 24 hours with 3 mice per group. The animals were euthanized by CO_2 asphyxiation. Upon cessation of breathing, the chest cavity was opened and blood sampled (200-500 µL) from the heart. Sampled blood was added to a heparinized microfuge tube and centrifuged for 10 minutes to separate plasma and blood cells. Plasma samples were treated with proteinase K containing buffer(100 mM NaCl, 10 mM tris (pH 8), 25mM EDTA, 10% SDS). A portion of the sample was to scintillant and counted. The remaining sample was resolved on a polyacrylamide gel and intact ribozyme bands were quantitated using a phosphorimager (molecular devices). The results are shown in figure **15.** Formulation of ribozyme with EPC:CHOL:DSPE:PEG C18 greatly enhances the circulation time of intact ribozyme in plasma. Twenty-four hours after an intravenous bolus injection of 2 mg/kg ribozyme formulated with EPC:CHOL:DSPE:PEG_{2000}, over 6% of the dose remained in the plasma. Average concentrations dropped from an average of 6631 ng/ml at 15 minutes to 2305 ng/ml at 24 hours. Since plasma concentrations were relatively high 24 hours after an injection, it can be assumed that the elimination half-life is on the order of hours if not days. In comparison, an intravenous bolus injection of 30 mg/kg is no longer detectable after approximately 3 hours. The elimination half-life of unformulated ribozyme is approximately 30 minutes in the mouse.

Example 21: Plasmid DNA Delivery into Cells in Culture

One day prior to transfection, target cells were plated to a final confluency of 50 to 60% on a 48 well plate. Cells types tested in serum free conditions are RT-4 (human bladder carcinoma), EJ (human bladder carcinoma), PC-3 prostate cancer cell line), and MCF-7 (breast cancer cell line). The following cell types were tested in the presence of 10% serum in the media: RT-4, PC-3 and MCF-7 cells. DNA (1 μg of pEGFP-C1 C-terminal protein fusion vector (Clontech)) was added to a polystyrene tube followed by the addition of 1 ml of desired media. Following agitation, the cationic lipid was added to the tube (1.25, 2.5, 5, or 10 μg lipid/μg DNA), incubated at room temperature for 15 minutes and then mixed by vortexing. Media from plated cells was aspirated and then washed with either serum free or normal growth media. 200μL of the DNA/cationic lipid mixture was added to each well of a 48 well plate. The cells were incubated at 37°C for 3 to 5 hours for serum-free uptake and 18 to 24 hours for uptake in the presence of serum. Fluorescent cells were then counted using fluorescence microscopy. The transfection rate was determined by comparing the number of fluorescence positive cells to the total number of cells in the microscope field.

Toxicity was determined by adding 5 μL of a 0.5 mg/ml stock solution of propidium iodine (PI) (Boehringer Mannheim) prior to examination by microscopy. Migration of the red dye into the nucleus of a cell indicated toxicity and loss of cell viability. The results of plasmid delivery in serum free media are shown in table VI. The results of plasmid delivery in the presence of serum is shown in table VII.

FACS (fluorescence activated cell sorting) analysis was performed on PC-3 cells using several formulations and the results are shown in table VIII. Transfection was achieved using the protocol described above with the cells being incubated with DNA for 4 hours in serum free conditions. Cells were trypsinized off the plate, collected in serum containing growth media, and spun down for 5 minutes at 800 RPM. The supernatant was removed and the cells were brought up in 500 μL of FAC buffer (4% FBS in Hank's balanced salt solution(HBSS)). 10 μL of 0.5 mg/ml of PI was added prior to FACS sorting. The results indicate that applicant's formulations improve delivery of macromolecules compared to other compounds which are commercially available.

EXAMPLE 22: Preparation of Cationic Lipids conjugated to polyethylene glycol via amide bond

Cationic lipid (100 mg), methoxypolyoxyethylenecarboxylic acid (725 mg), and 1,3- dicyclohexylcarbodiimide (DCC)(30 mg) were dissolved in chloroform (30 mL) and the solution was allowed to react at 50°C overnight. The reaction mixture was filtered and hexane was added to the filtrate for purification by precipitation. The product (*N*'-palmityl-*N*'-oleyl-α-amino,γ-PEGamino-glycinamide) was re-precipitated using the same procedure and then dried *in vacuo* to obtain a PEG-conjugated lipid. In addition to carboxy-terminated PEG, N-hydroxysuccinimdie activated ester of PEG can be utilized in the above conjugation procedures.

Conjugation may also be carried out by formation of a carbamate bond. The reaction would be initiated by reacting imidazolylcarbonate activated hydroxy-terminated methoxypolyethylene glycol with amino groups of cationic lipids described above. The methods described herein are not limiting. Those skilled in the art will recognize that PEG can be readily conjugated to cationic lipids using techniques known in the art and are within the scope of this invention.

Example 23: Transfection of Jurkat cells with Fluorescein conjugated oligonucleotide

The ability of cytofectins of the invention to transfect suspension cells was tested in Jurkat cells. These cells were grown and maintained in RPMI-1640 culture medium supplemented with 10% fetal bovine serum and glutamine (Life Technologies). The cells were diluted in culture medium to $5x10^5$ cells/ml, and 80 μl (40,000 cells) was transferred into each well of a 96-well culture dish. A fluorescein-conjugated, nuclease resistant 23-mer oligonucleotide of randomized sequence was chemically synthesized for transfection. Additional oligonucleotides were synthesized using standard methodologies (Wincott *et al.,* supra).

The fluorescein-conjugated oligonucleotides were premixed with cytofectins (Table IX) at 5X concentrations (20 μl volume) and added to wells containing cells in 80 μl medium (total incubation volume=100 μl). In typical experiments, 100 nM oligonucleotide (final concentration) was mixed with 2.5, 5, or 10 μg/ml of each delivery vehicle. In some experiments, culture plates were immediately placed in a humidified incubator overnight (18-24 h). In other experiments, the plates containing

cells and oligonucleotide-lipid complexes were first spun for 40 minutes at room temperature and then incubated overnight. The following day, the cells were gently spun down and the incubation medium was replaced with normal growth medium.

Delivery of oligonucleotides into cells was determined by fluorescence microscopy. The cellular nuclei were examined to identify whether fluorescence was emitted from within the nuclear envelope. Nuclear delivery indicated that the oligonucleotide had permeated across the topological membrane barrier and entered the cytosol followed by migration to nucleus. Alternatively, *punctate* perinuclear pattern of fluorescence around the nucleus indicated that the oligonucleotide, internalized by an endocytic mechanism, remained sequestered within the endosomal vesicles. Jurkat cells are small and round and the nucleus occupies much of the cellular volume. Nuclear as well as perinuclear cytoplasmic labeling was observed in most cells. The efficiency of delivery was estimated by calculating the number of cells with green nuclei as a percentage of the total number of cells in the field, as observed by phase contrast microscopy. The acute toxicity of the treatments was assessed by adding propidium iodide (1 μg/ml) to each well. Damaged cells with compromised membranes internalized propidium iodide and could be easily identified by red fluorescence in the nucleus.

Three cytofectins (Formulaiton ID Nos: 345, 323, and 333) were found to transfect the oligonucleotide at a very efficient rate. Depending on the formulation, delivery into the nuclei of jurkat cells ranged from 40 to 80% (Table IX).

Example 24: Inhibition of Inosine monophosphate dehydrogenase (IMPDH) Using Nucleic Acid Molecules

The cytofectins identified using the delivery screen described in Example 23 were then used in efficacy assays. An antisense molecule directed against the mRNA for IMPDH, an essential enzyme involved in nucleic acid production, was formulated with the delivery vehicle and administered to Jurkat cells seeded in 96-well plates as described above. Assays were done in triplicate wells and cells were incubated for 24 h. A random sequence antisense molecule of the same length and containing the same type of linkages as the IMPDH binding antisense molecule was used as control. A quantitative Taqman assay (Perkin Elmer) was developed to measure the changes in

IMPDH mRNA levels relative to actin, a constitutively expressed housekeeping gene. RNA from treated or untreated cells was extracted and then analyzed by Taqman. All three of the cytofectins tested were able to deliver sufficient antisense molecule to inhibit the expression of IMPDH-2 (Figures 16-18).

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and

variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group. Other embodiments are within the claims that follow.

TABLE I

Characteristics of naturally occurring ribozymes

Group I Introns

- Size: ~150 to >1000 nucleotides.
- Requires a U in the target sequence immediately 5' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site.
- Reaction mechanism: attack by the 3'-OH of guanosine to generate cleavage products with 3'-OH and 5'-guanosine.
- Additional protein cofactors required in some cases to help folding and maintainance of the active structure.
- Over 300 known members of this class. Found as an intervening sequence in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.
- Major structural features largely established through phylogenetic comparisons, mutagenesis, and biochemical studies [,1].
- Complete kinetic framework established for one ribozyme [2,3,4,5].
- Studies of ribozyme folding and substrate docking underway [6,7,8].
- Chemical modification investigation of important residues well established [9,10].
- The small (4-6 nt) binding site may make this ribozyme too non-specific for targeted RNA cleavage, however, the Tetrahymena group I intron has been used to repair a "defective" β-galactosidase message by the ligation of new β-galactosidase sequences onto the defective message [11].

RNAse P RNA (M1 RNA)

- Size: ~290 to 400 nucleotides.
- RNA portion of a ubiquitous ribonucleoprotein enzyme.
- Cleaves tRNA precursors to form mature tRNA [12].
- Reaction mechanism: possible attack by M^{2+}-OH to generate cleavage products with 3'-OH and 5'-phosphate.
- RNAse P is found throughout the prokaryotes and eukaryotes. The RNA subunit has been sequenced from bacteria, yeast, rodents, and primates.
- Recruitment of endogenous RNAse P for therapeutic applications is possible through hybridization of an External Guide Sequence (EGS) to the target RNA [13,14]
- Important phosphate and 2' OH contacts recently identified [15,16]

Group II Introns

- Size: >1000 nucleotides.
- Trans cleavage of target RNAs recently demonstrated [17,18].

- Sequence requirements not fully determined.
- Reaction mechanism: 2′-OH of an internal adenosine generates cleavage products with 3′-OH and a "lariat" RNA containing a 3′-5′ and a 2′-5′ branch point.
- Only natural ribozyme with demonstrated participation in DNA cleavage [19,20] in addition to RNA cleavage and ligation.
- Major structural features largely established through phylogenetic comparisons [21].
- Important 2′ OH contacts beginning to be identified [22]
- Kinetic framework under development [23]

Neurospora VS RNA

- Size: ~144 nucleotides.
- Trans cleavage of hairpin target RNAs recently demonstrated [24].
- Sequence requirements not fully determined.
- Reaction mechanism: attack by 2′-OH 5′ to the scissile bond to generate cleavage products with 2′,3′-cyclic phosphate and 5′-OH ends.
- Binding sites and structural requirements not fully determined.
- Only 1 known member of this class. Found in Neurospora VS RNA.

Hammerhead Ribozyme

(see text for references)

- Size: ~13 to 40 nucleotides.
- Requires the target sequence UH immediately 5' of the cleavage site.
- Binds a variable number nucleotides on both sides of the cleavage site.
- Reaction mechanism: attack by 2′-OH 5′ to the scissile bond to generate cleavage products with 2′,3′-cyclic phosphate and 5′-OH ends.
- 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent.
- Essential structural features largely defined, including 2 crystal structures [25,26]
- Minimal ligation activity demonstrated (for engineering through *in vitro* selection) [27]
- Complete kinetic framework established for two or more ribozymes [28].
- Chemical modification investigation of important residues well established [29].

Hairpin Ribozyme

- Size: ~50 nucleotides.
- Requires the target sequence GUC immediately 3' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site and a variable number to the 3'-side of the cleavage site.
- Reaction mechanism: attack by 2′-OH 5′ to the scissile bond to generate cleavage products with 2′,3′-cyclic phosphate and 5′-OH ends.
- 3 known members of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent.
- Essential structural features largely defined [30,31,32,33]

- Ligation activity (in addition to cleavage activity) makes ribozyme amenable to engineering through *in vitro* selection [34]
- Complete kinetic framework established for one ribozyme [35].
- Chemical modification investigation of important residues begun [36,37].

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [38].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [39].
- Reaction mechanism: attack by 2′-OH 5′ to the scissile bond to generate cleavage products with 2′,3′-cyclic phosphate and 5′-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [40]

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Table 1

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Table II: Cationic Lipid Formulations and Cellular Uptake

Compound Name	Cationic Lipid Name	Cationic lipid:DOPE (molar ratio)	Formulation Name	Dose (µg/ml)	Nuclear Localization 0=none; 5=all	Cytoplasmic Localization
DS46596a	Arg-chol-2 BOCs	1:0	nc99	5	1	punct.
				10	1	punct.
		1:1	nc25	5	2-3	punct.
				10	3-4	punct.
		2:1	nc26	5	2-3	punct.
				10	3	punct.
		3:1	nc27	5	2-4	punct.
				10	2-4	punct.
DS46596b	Arg-chol-3 BOCs	1:0	nc100	5	1	some clumps
				10	1	some clumps
		1:1	nc4	5	4	punct.
				10	3	punct.
		2:1	nc5	5	1	punct.
				10	1	punct.
		3:1	nc6	5	1	punct.
				10	1	punct.
PHF55933	Chol-methyl-					
		1:1	nc13	5	0	bright punct.
				10	0	very bright
		2:1	nc14	5	0-1	bright punct.
				10	0	bright punct.
		3:1	nc15	5	0	bright punct.
				10	0	very bright
AK52450	Dimyristoyl-pyridoxal	1:0	nc108	5	0	few clumps
				10	0	
		1:1	nc16	5	0	
				10	0	
		2:1	nc17	5	0	
				10	0	
		3:1	nc18	5	0	
				10	0	

Table II

JA59311	Palmitoyl oleoyl glycyldiamino butyric acid	1:0	nc101	5	0-1	some punct.
				10	3	punct.
		1:1	nc19	5	2	some punct.
				10	1	bright punct
		2:1	nc20	5	1, faint	punct.
				10	3, var. bright.	punct.
		3:1	nc21	5	3	bright punct.
				10	3	bright punct.
PH55938	Lys-chol.	1:0	nc104	5	0-1,few brt.	no punct.
				10	0-1	clumps.
		1:1	nc22	5	0-1	bright punct
				10	0-1	bright punct
		2:1	nc23	5	0	bright punct
				10	0	bright punct
		3:1	nc24	5	0	punctate
				10	0	bright punct.
AK52465	*N*-cholesteryl-pyridoxamine	1:0	nc109	5	0-1, very faint	
				10	1, very faint	some clumps
		1:1	nc28	5	0	
				10	0-1	
		2:1	nc29	5	0	
				10	0	
		3:1	nc30	5	0	
				10	0	
AK52468	Beta-alanine palm.oleoyl-amide	1:0	nc110	5	3, bright	punct.
				10	3-4, bright	punct.
		1:1	nc31	5	0	punct.
				10	0	punct.
		2:1	nc32	5	0-1	punct.
				10	2-3	punct.
		3:1	nc33	5	0-1	punct.
				10	3-4	punct.
AK52471	dipalmitoyl-deoxy-aminoethyl-carboxamido-Uridine	1:0	nc111	5	0	punct.
				10	0-1	punct./clumps

Table II

		1:1	nc34	5	0	clumps
				10	0	clumps
		2:1	nc35	5	0	clumps
				10	0	clumps
		3:1	nc36	5	0	clumps
				10	0	clumps
AK52474	palmitoyl-oleoyl-carboxamido-ethyl-pyridoxamine	1:0	nc112	5	0	
				10	0	
		1:1	nc37	5	0	faint punct.
				10	0-1	faint punct.
		2:1	nc38	5	0	faint
				10	0	faint punct
		3:1	nc39	5	0	
				10	0	faint punct.
PH55939		1:0	nc105	5	0-1	punct.
				10	0-1	punct.
		1:1	nc40	5	0	punct.
				10	0	punct.
		2:1	nc41	5	0-1	punct/vac.
				10	2-3	punct
		3:1	nc42	5	0-1	punct.
				10	0-1	bright punct.
PH55941		1:0	nc106	5	0-1	punct., clumps
				10	0-1	brt. pnc., clumps
		1:1	nc43	5	0-1	punct.
				10	2-3	heavy punct.
		2:1	nc44	5	0-1	heavy punct.
				10	0-1	heavy punct.
		3:1	nc45	5	0-1	punct., clumps
				10	0-1	punct., clumps
PH55942		1:0	nc107	5	0-1	bright punct.
				10	0-1	bright punct.
		1:1	nc46	5	0-1	punct.
				10	3	punct.
		2:1	nc47	5	0-1	punct.
				10	0-1	bright punct.
		3:1	nc48	5	0-1	punct.
				10	0-1	bright punct.

Table II

JA59312	plamitoyl oleoyl glycylamino-guanyl diamino-butyric acid	1:0	nc102	5	1	bright punct.
				10	4	bright punct.
		1:1	nc49	5	3	punct.
				10	4	bright punct.
		2:1	nc50	5	3-4	bright punct.
				10	4	bright punct.
		3:1	nc51	5	4-5	bright punct.
				10	5	bright punct.
JA59314		1:0	nc103	5	0-1	punct.
				10	0-1	heavy punct.
		1:1	nc52	5	0	punct.
				10	0	punct.
		2:1	nc53	5	0	punct.
				10	0	punct.
		3:1	nc54	5	0	faint punct.
				10	0	faint punct.
AK52475	dipalmitoyl-deoxy-guanidino-ethyl-carboxamido-uridine	1:0	nc96	5	0-1	some clumps
				10	0-1	clumps
		1:1	nc84	5	0	clumps/st. to gl.
				10	0	clumps/st. to gl.
		2:1	nc85	5	0	clumps/st. to gl.
				10	0	clumps/st. to gl.
		3:1	nc86	5	0	clumps/st. to gl.
				10	0	clumps/st. to gl.
JA59316	palmityl-oleoyl-diamino-butyric acid	1:0	nc97	5	0	heavy punct.
				10	0	heavy punct.
		1:1	nc87	5	0	lt. punct./sticky
				10	0	lt. punct./sticky

Table II

		2:1	nc88	5	0	lt. punct./sticky
				10	0	lt. punct./sticky
		3:1	nc89	5	0	sticky/out.
				10	0	sticky/out
JA59317	palmity-oleoyl-guanidine	1:0	nc98	5	0-1	faint punct.
				10	0-1	punct.
		1:1	nc90	5	1-2	punct.
				10	2-3	punct.
		2:1	nc91	5	1-2	punct.
				10	1-2	punct.
		3:1	nc92	5	1	punct.
				10	1	punct.

Table III

Cells	Description	Optimal cationic lipids (>50% nuclear uptake)
HS27	human foreskin fibroblasts	nc49, nc50
HUVEC	human umbilical vein endothelial	nc51, nc26
RAOSMC	rat aortic smooth muscle	nc102, nc49
SK-N-SH	human neuroblastoma	nc21, nc25
EJ	human bladder carcinoma	nc21, nc49
RT-4	human bladder carcinoma	nc49, nc21
MCF-7	human breast carcinoma	nc21
FEM	human melanoma	nc21
1205	human melanoma	nc49
PC-3	human prostate carcinoma	nc49, nc21
LN-CAP	human prostate carcinoma	nc110, nc21

Table III: Lipid-mediated delivery of ribozymes to various cell lines. Cells were treated with 100 nM fluorescein-conjugated ribozymes formulated with a panel of cationic lipids (selected from nc21, nc25, nc26, nc49, nc51, nc102, nc110; see Methods). Subcellular distribution of the ribozyme was determined by fluorescence microscopy. Presence of fluorescence in the nucleus indicated that the ribozyme had been transported across the cell membrane (unconjugated fluorescein does not remain in the nucleus). Lipid formulations that led to high nuclear delivery with no significant toxicity are shown.

Table IV

Cells	Description	Optimal cationic lipids (% of cells with GFP)
SK-N-SH	human neuroblastoma	nc49 (40%) nc101 (25%) nc110 (20%) nc32 (15%)
RASMC	rat aortic smooth muscle	nc110 (~5%)
RT-4	human bladder carcinoma	nc101 (60%) nc19 (20%) nc110 (20%)
HS27	human foreskin fibroblasts	nc101 (~5%)

Table IV: Lipid-mediated delivery of plasmids to various cell lines. Cells were treated with 0.1-1 μg/ml of a green fluorescent protein (GFP) expression plasmid formulated with 2.5-15 μg/ml of selected lipid formulations (Table II), as described (Methods). The expression of GFP was monitored by fluorescence microscopy ~20 hours after transfection. Formulations that resulted in GFP expression by ~5% or more of the cells are indicated.

Table V

Lipid (formulation)	Nuclear Uptake?	% Inhibition
JA59312 (nc102)	Y >50%	40%
JA59312 (nc49)	Y >40%	38%
JA59317 (nc98)	Y >10%	23%
JA59311 (nc101)	Y >5%	21%
JA59311 (nc20)	Y>10%	0%
PH55942 (nc48)	N (punct.)	18%
AK52468 (nc33)	N (punct.)	16%
JA59316 (nc97)	N (punct.)	0%
PHF55933 (nc13)	N	0%
PZH55938 (nc22)	N	0%

Table V. Inhibition of cell proliferation by different ribozyme formulations and correlation with cellular and nuclear delivery. An anti-myb ribozyme and its inactive version (control) were formulated with various lipids (Table II) and administered to rat smooth muscle cells as described (Methods). The relative activity of the active vs. inactive ribozyme is shown (% inhibition of proliferation). In parallel experiments, cells were treated with identical formulations of a fluorescein-conjugated ribozyme and its subcellular localization was observed by fluorescence microscopy (Y, nuclear delivery (% of positive cells); N, no visible nuclear delivery; punct., punctate cytoplasmic fluorescence). In general, formulations that led to improved delivery of the ribozyme to the cell and the nucleus also led to increased efficacy.

Table VI. Delivery of Green Fluorescent Protein Containing Plasmid into Cells in Culture

Cell type	Formulation name	Compounds	Transfection rate	Optimal lipid dose	Toxicity
PC-3	nc 19	JA 59311:DOPE at 1:1	40-64%	2.5µg/ml	<10%
PC-3	nc 20	JA 59311:DOPE at 2:1	40-50%	2.5µg/ml	<5%
PC-3	nc 101B	JA 59311	70 -75%	5µg/ml	<10%
PC-3	nc 102	JA 59312	30-45%	2.5µg/ml	10%
PC-3	nc 110D	AK 52468	60%	2.5µg/ml	5%
PC-3	nc 122	JA 59317 & JA 59311-2:1	55-65%	2.5µg/ml	5-13%
PC-3	nc 123	JA 59317 & JA 59311-3:1	30-50%	2.5µg/ml	5%
PC-3	nc 128	JA 59317 & PH 55942-2:1	35-70%	2.5µg/ml	<3-15%
PC-3	nc 144	JA 59311 & JA 59312-2:1	40-60%	2.5µg/ml	<3-10%
PC-3	nc 145	JA 59311 & JA 59312-3:1	50-70%	2.5µg/ml	<3-20%
PC-3	nc 146B	JA 59311 & AK 52468-1:1	40-69%	2.5µg/ml	<3-8%
PC-3	nc 148B	JA 59311 & AK 52648-3:1	25-70%	2.5µg/ml	3-20%
PC-3	nc 156B	JA 59312 & JA 59311 at 3:1	40-65%	1.5µg/ml	<5%
PC-3	nc 168	AK 52468 & JA 59312 at 2:1	33-75%	2.5µg/ml	5-10%
PC-3	nc 169	AK 52468 & JA 59312 at 3:1	50-79%	2.5µg/ml	5-10%
RT-4	nc 101B	JA 59311	30 to 68%	5µg/ml	<10%
RT-4	nc 121	JA 59311 & PH 55942-3:1	8-30%	5µg/ml	<5%
RT-4	nc 122	JA 59317 & JA 59311-2:1	20%	2.5µg/ml	<6%
RT-4	nc 144	JA 59311 & JA 59312-2:1	50%	10µg/ml	<10%
RT-4	nc 145	JA 59311 & JA 59312-3:1	50%	10µg/ml	<10%
EJ cells	nc 19	JA 59311:DOPE at 1:1	40%	5µg/ml	10%
EJ cells	nc 20	JA 59311:DOPE at 2:1	20%	5µg/ml	5-10%
EJ cells	nc 101B	JA 59311	25-35%	10µg/ml	5%
EJ cells	nc 110D	AK 52468	30%	5µg/ml	<5%
EJ cells	nc 122	JA 59317 & JA 59311 -2:1	35%	5µg/ml	10%
EJ cells	nc 144	JA 59311 & JA 59312-2:1	40%	2.5µg/ml	<3%
EJ cells	nc 145	JA 59311 & JA 59312-3:1	35%	5µg/ml	5%
MCF-7	nc 110D	AK 52468	20-30%	2.5µg/ml	4-20%
MCF-7	nc 121	JA 59311 & PH 55942-3:1	10-40%	5µg/ml	8-10%
MCF-7	nc 146B	JA 59311 & AK 52468 at 1:1	50%	5µg/ml	15%
COS-7	nc21	JA59311: DOPE (3:1)	40%	2.5µg/ml	<5%
COS-7	nc 101	JA 59311	50%	5µg/ml	<5%
COS-7	nc 110	AK 52468	40%	5µg/ml	<5%
HeLa	nc 145	JA 59311 & JA 59312 (3:1)	40-50%	2.5µg/ml	<5%

Cell type	Formulation name	Compounds	Transfection rate	Optimal lipid dose	Toxicity
RT-4	nc 193	JA 59311-1:Tween80 at 1:1	5-40%	10μg/ml	<1-40%
RT-4	nc 194	JA 59311-1:Tween 80 at 2:1	10-20%	10μg/ml	<1-10%
RT-4	nc 195	JA 59311-1:Tween80 at 3:1	10-40%	10μg/ml	5-10%
RT-4	nc 196	JA 59311-1:Tween80 at 4:1	20%	5μg/ml	<3%
RT-4	nc 220	JA 59349:Tween80 at 6:1	15-40%	10μg/ml	5-10%
PC-3	nc 110D	AK 52468	40%	5μg/ml	8%
PC-3	nc 194	JA 59311-1:Tween 80 at 2:1	20-45%	10μg/ml	<1-10%
PC-3	nc 195	JA 59311-1:Tween80 at 3:1	60%	10μg/ml	10%
PC-3	nc 218	JA 59311-1:Tween80 at 3:1	40-70%	10μg/ml	5-40%
PC-3	nc 219	JA 59349:Tween80 at 4:1	60-75%	10μg/ml	10-20%
MCF-7	nc 110D	AK 52468	50%	2.5μg/ml	20%

Table VII. Delivery of Green Fluorescent Protein Containing Plasmid into Cells in Culture in the Presence of 10% serum

Formulation	Lipid Dose	Plasmid DNA	% GFP positive
Lipofectamine*	1.25μg/ml	1μg/ml	3
	2.5μg/ml	1μg/ml	24
PFX-6 *	1.25μg/ml	1μg/ml	4
	2.5μg/ml	1μg/ml	15
nc 146	1.25μg/ml	1μg/ml	40
	2.5μg/ml	1μg/ml	65
nc 148	1.25μg/ml	1μg/ml	41
	2.5μg/ml	1μg/ml	56
nc 156	1.25μg/ml	1μg/ml	45
	2.5μg/ml	1μg/ml	71
nc 169	1.25μg/ml	1μg/ml	53
	2.5μg/ml	1μg/ml	72

*-commercially available cationic lipids

Table VIII. FACS analysis of plasmid delivery into PC-3 cells of several cationic lipids after 4 hours in serum free media. GFP=Green Fluorescent Protein.

Table IX: Lipid Formulations

Formulation ID No.	Lipid ID No. ratios	Lipid name ratios	mass ratios
282	700 / 747	JA59311 / chol-linoleate	3 / 1
283	700 / 747	JA59311 / chol-linoleate	1 / 1
284	726 / 743	DPPE / JA73852	1 / 2
285	709 / 727	DS46596a / DEPE	3 / 1
286	719 / 726	AK52468 / DPPE	1 / 1
287	727 / 749	DEPE / EP-G-DABA mix	1 / 1
288	743 / 747	JA73852 / chol-linoleate	1 / 1
289	701 / 745	JA59312 / cholesterol	2 / 1
290	700 / 746	JA59311 / chol-linolelaidate	2 / 1
291	705 / 722	JA59396 / DOPE	2.98 / 1
292	732 / 747	PH55942 / chol-linoleate	1 / 1
293	722 / 742	DOPE / JA73851	1 / 3
294	700 / 727	JA59311 / DEPE	2 / 1
295	700 / 722 / 745	JA59311 / DOPE / cholesterol	1 / 1 / 1
296	700 / 705 / 722	JA59311 / JA59396 / DOPE	9.01 / 1 / 3.36
297	701 / 746	JA59312 / chol-linolelaidate	2 / 1
298	700 / 727	JA59311 / DEPE	1 / 1
299	742 / 745	JA73851 / cholesterol	1 / 1
300	705 / 726	JA59396 / DPPE	1 / 1
301	709 / 725	DS46596a / DLPE	3 / 1
302	726 / 732	DPPE / PH55942	1 / 2
303	723 / 736	Tween 80 / JA59350	1 / 1
304	723 / 736	Tween 80 / JA59350	1 / 6
305	701 / 727	JA59312 / DEPE	3.08 / 1
306	727 / 742	DEPE / JA73851	1 / 2
307	743 / 746	JA73852 / chol-linolelaidate	3 / 1

308	701 / 727	JA59312 / DEPE	2.06 / 1
309	726 / 744	DPPE / JA73853	1 / 1
310	725 / 750	DLPE / EP-G-AGBA mix	1 / 2.05
311	722 / 743	DOPE / JA73852	1 / 3
312	700 / 726	JA59311 / DPPE	2.99 / 1
313	727 / 749	DEPE / EP-G-DABA mix	1 / 2
314	701 / 745	JA59312 / cholesterol	3 / 1
315	705 / 725	JA59396 / DLPE	1 / 1
316	725 / 732	DLPE / PH55942	1 / 1
317	727 / 744	DEPE / JA73853	1 / 1
318	723 / 736	Tween 80 / JA59350	1 / 2
319	744 / 746	JA73853 / chol-linolelaidate	3 / 1
320	723 / 737	Tween 80 / JA59351	1 / 1
321	727 / 743	DEPE / JA73852	1 / 2
322	701 / 746	JA59312 / chol-linolelaidate	1 / 1
323	726 / 749	DPPE / EP-G-DABA mix	1 / 2.99
324	709 / 747	DS46596a / chol-linoleate	1 / 1
325	723 / 737	Tween 80 / JA59351	1 / 2
326	701 / 746	JA59312 / chol-linolelaidate	3 / 1
327	705 / 725	JA59396 / DLPE	2 / 1
328	732 / 747	PH55942 / chol-linoleate	2 / 1
329	701 / 747	JA59312 / chol-linoleate	2 / 1
330	700 / 726 / 747	JA59311 / DPPE / chol-linoleate	3 / 1 / 1
331	743 / 747	JA73852 / chol-linoleate	3 / 1
332	705 / 722	JA59396 / DOPE	1 / 1
333	701 / 726	JA59312 / DPPE	2.06 / 1
334	705	JA59396	1
335	727 / 732	DEPE / PH55942	1 / 3
336	722 / 744	DOPE / JA73853	1 / 1
337	709 / 747	DS46596a / chol-linoleate	2 / 1

338	700 / 726	JA59311 / DPPE	2 / 1
339	723 / 737	Tween 80 / JA59351	1 / 6
340	742 / 746	JA73851 / chol-linolelaidate	3 / 1
341	727 / 750	DEPE / EP-G-AGBA mix	1 / 2.06
342	700 / 705	JA59311 / JA59396	1 / 9
343	700 / 705	JA59311 / JA59396	8 / 2
344	701 / 725	JA59312 / DLPE	2.05 / 1
345	726 / 749	DPPE / EP-G-DABA mix	1 / 2
346	725 / 750	DLPE / EP-G-AGBA mix	1 / 1.03
347	743 / 747	JA73852 / chol-linoleate	2 / 1
348	742 / 747	JA73851 / chol-linoleate	2 / 1
349	709 / 727	DS46596a / DEPE	1 / 1
350	700 / 722 / 745	JA59311 / DOPE / cholesterol	3 / 1 / 1
351	744 / 747	JA73853 / chol-linoleate	2 / 1
352	700 / 745	JA59311 / cholesterol	2 / 1
353	722 / 743	DOPE / JA73852	1 / 1
354	719 / 727	AK52468 / DEPE	1 / 1
355	700 / 705	JA59311 / JA59396	2 / 8
356	727 / 750	DEPE / EP-G-AGBA mix	1 / 1.03
357	726 / 749	DPPE / EP-G-DABA mix	1 / 1
358	742 / 745	JA73851 / cholesterol	3 / 1
359	723 / 738	Tween 80 / JA59352	1 / 1
360	705 / 726	JA59396 / DPPE	2.98 / 1
361	701 / 726	JA59312 / DPPE	3.09 / 1
362	709 / 727	DS46596a / DEPE	2 / 1
363	701 / 726	JA59312 / DPPE	1.03 / 1
364	701 / 747	JA59312 / chol-linoleate	1 / 1
365	705 / 722	JA59396 / DOPE	3 / 1
366	723 / 739	Tween 80 / JA59353	1 / 1
367	723 / 739	Tween 80 / JA59353	1 / 6

368	744 / 747	JA73853 / chol-linoleate	1 / 1
369	709 / 747	DS46596a / chol-linoleate	3 / 1
370	700 / 726 / 745	JA59311 / DPPE / cholesterol	1 / 1 / 1
371	732 / 745	PH55942 / cholesterol	2 / 1
372	705 / 726	JA59396 / DPPE	2 / 1
373	705 / 722	JA59396 / DOPE	2 / 1
374	742 / 747	JA73851 / chol-linoleate	3 / 1
375	701 / 745	JA59312 / cholesterol	1 / 1
376	726 / 743	DPPE / JA73852	1 / 1
377	709 / 726	DS46596a / DPPE	3 / 1
378	723 / 736	Tween 80 / JA59350	1 / 4
379	719 / 725	AK52468 / DLPE	2 / 1
380	709 / 726	DS46596a / DPPE	1 / 1
381	700 / 727	JA59311 / DEPE	2.99 / 1
382	700 / 705	JA59311 / JA59396	6 / 4
383	700 / 705 / 722	JA59311 / JA59396 / DOPE	8.01 / 2 / 3.36
384	744 / 747	JA73853 / chol-linoleate	3 / 1
385	742 / 747	JA73851 / chol-linoleate	1 / 1
386	705 / 727	JA59396 / DEPE	3 / 1
387	700 / 747	JA59311 / chol-linoleate	2 / 1
388	701 / 747	JA59312 / chol-linoleate	3 / 1
389	726 / 743	DPPE / JA73852	1 / 3
390	732 / 747	PH55942 / chol-linoleate	3 / 1
391	700 / 705 / 722	JA59311 / JA59396 / DOPE	5.01 / 5 / 3.36
392	719 / 726	AK52468 / DPPE	3 / 1
393	719 / 726	AK52468 / DPPE	2 / 1
394	705 / 727	JA59396 / DEPE	1 / 1
395	700 / 726 / 745	JA59311 / DPPE / cholesterol	3 / 1 / 1
396	700 / 725	JA59311 / DLPE	2.01 / 1
397	700 / 705	JA59311 / JA59396	1 / 1

398	700 / 705 / 722	JA59311 / JA59396 / DOPE	6.01 / 4 / 3.36
399	742 / 746	JA73851 / chol-linolelaidate	1 / 1
400	700 / 705 / 722	JA59311 / JA59396 / DOPE	7.01 / 3 / 3.36
401	709 / 726	DS46596a / DPPE	2 / 1
402	744 / 745	JA73853 / cholesterol	1 / 1
403	722 / 742	DOPE / JA73851	1 / 2
404	744 / 745	JA73853 / cholesterol	2 / 1
405	722 / 744	DOPE / JA73853	1 / 2
406	723 / 738	Tween 80 / JA59352	1 / 6
407	700 / 725	JA59311 / DLPE	1 / 1
408	725 / 750	DLPE / EP-G-AGBA mix	1 / 3.09
409	709 / 725	DS46596a / DLPE	2 / 1
410	725 / 732	DLPE / PH55942	1 / 2
411	700 / 705	JA59311 / JA59396	4 / 6
412	700 / 705 / 722	JA59311 / JA59396 / DOPE	3 / 7 / 3.35
413	700 / 705 / 722	JA59311 / JA59396 / DOPE	2 / 8 / 3.36
414	700 / 705 / 722	JA59311 / JA59396 / DOPE	1.31 / 9 / 3.36
415	723 / 738	Tween 80 / JA59352	1 / 4
416	727 / 744	DEPE / JA73853	1 / 2
417	719 / 725	AK52468 / DLPE	1 / 1
418	700 / 745	JA59311 / cholesterol	3 / 1
419	726 / 732	DPPE / PH55942	1 / 1
420	727 / 732	DEPE / PH55942	1 / 2
421	744 / 745	JA73853 / cholesterol	3 / 1
422	709 / 745	DS46596a / cholesterol	3 / 1
423	705 / 727	JA59396 / DEPE	2 / 1
424	727 / 743	DEPE / JA73852	1 / 3
425	701 / 725	JA59312 / DLPE	3.09 / 1
426	727 / 732	DEPE / PH55942	1 / 1
427	723 / 738	Tween 80 / JA59352	1 / 2

428	743 / 745	JA73852 / cholesterol	2 / 1
429	743 / 746	JA73852 / chol-linolelaidate	2 / 1
430	700 / 705	JA59311 / JA59396	3 / 7
431	700 / 746	JA59311 / chol-linolelaidate	3 / 1
432	722 / 743	DOPE / JA73852	1 / 2
433	727 / 750	DEPE / EP-G-AGBA mix	1 / 3.08
434	700 / 725	JA59311 / DLPE	3 / 1
435	732 / 745	PH55942 / cholesterol	3 / 1
436	727 / 743	DEPE / JA73852	1 / 1
437	743 / 745	JA73852 / cholesterol	3 / 1
438	719 / 727	AK52468 / DEPE	2.99 / 1
439	725 / 732	DLPE / PH55942	1 / 3
440	725 / 749	DLPE / EP-G-DABA mix	1 / 3
441	743 / 746	JA73852 / chol-linolelaidate	1 / 1
442	743 / 745	JA73852 / cholesterol	1 / 1
443	726 / 744	DPPE / JA73853	1 / 3
444	727 / 742	DEPE / JA73851	1 / 3
445	744 / 746	JA73853 / chol-linolelaidate	2 / 1
446	701 / 725	JA59312 / DLPE	1.03 / 1
447	705 / 725	JA59396 / DLPE	2.99 / 1
448	709 / 745	DS46596a / cholesterol	1 / 1
449	700 / 705	JA59311 / JA59396	9 / 1
450	709 / 725	DS46596a / DLPE	1 / 1
451	742 / 745	JA73851 / cholesterol	2 / 1
452	725 / 749	DLPE / EP-G-DABA mix	1 / 2.01
453	726 / 742	DPPE / JA73851	1 / 3
454	726 / 744	DPPE / JA73853	1 / 2
455	722 / 742	DOPE / JA73851	1 / 1
456	732 / 745	PH55942 / cholesterol	1 / 1
457	726 / 742	DPPE / JA73851	1 / 1

458	700 / 746	JA59311 / chol-linolelaidate	1 / 1
459	700 / 705	JA59311 / JA59396	7 / 3
460	700	JA59311	1
461	725 / 749	DLPE / EP-G-DABA mix	1 / 1
462	727 / 749	DEPE / EP-G-DABA mix	1 / 2.99
463	719 / 725	AK52468 / DLPE	3 / 1
464	727 / 744	DEPE / JA73853	1 / 3
465	700 / 705 / 722	JA59311 / JA59396 / DOPE	4.06 / 6 / 3.4
466	700 / 722	JA59311 / DOPE	2 / 1
467	744 / 746	JA73853 / chol-linolelaidate	1 / 1
468	723 / 739	Tween 80 / JA59353	1 / 2
469	727 / 742	DEPE / JA73851	1 / 1
470	723 / 739	Tween 80 / JA59353	1 / 4
471	723 / 737	Tween 80 / JA59351	1 / 4
472	700 / 722 / 745	JA59311 / DOPE / cholesterol	2 / 1 / 1
473	700 / 726 / 747	JA59311 / DPPE / chol-linoleate	2 / 1 / 1
474	726 / 732	DPPE / PH55942	1 / 3
475	726 / 742	DPPE / JA73851	1 / 2
476	700 / 745	JA59311 / cholesterol	1 / 1
477	719 / 727	AK52468 / DEPE	2 / 1
478	700 / 726 / 745	JA59311 / DPPE / cholesterol	2 / 1 / 1
479	700 / 726	JA59311 / DPPE	1 / 1
480	700 / 722	JA59311 / DOPE	2.99 / 1
481	722 / 744	DOPE / JA73853	1 / 3
482	701 / 727	JA59312 / DEPE	1.03 / 1
483	700 / 722	JA59311 / DOPE	1 / 1
484	709 / 745	DS46596a / cholesterol	2 / 1
485	700 / 726 / 747	JA59311 / DPPE / chol-linoleate	1 / 1 / 1
486	719 / 752	AK52468 / JA94882	1 / 2
487	700 / 722	JA59311 / DOPE	2 / 1

488	751 / 752	JA94881 / JA94882	1 / 1
489	701 / 726	JA59312 / DPPE	1.5 / 1
490	751 / 752	JA94881 / JA94882	0.2 / 1
491	752 / 753	JA94882 / JA10334	0.5 / 1
492	751 / 753	JA94881 / JA10334	1 / 1
493	726 / 751	DPPE / JA94881	1 / 2
494	701 / 726	JA59312 / DPPE	1.8 / 1
495	751 / 753	JA94881 / JA10334	0.5 / 1
496	719 / 752	AK52468 / JA94882	1 / 3
497	751 / 753	JA94881 / JA10334	2 / 1
498	700 / 722	JA59311 / DOPE	1 / 1
499	700 / 722	JA59311 / DOPE	3 / 1
500	753	JA10334	1
501	726 / 752	DPPE / JA94882	1 / 2
502	719 / 751	AK52468 / JA94881	1 / 2
503	751 / 753	JA94881 / JA10334	0.2 / 1
504	700 / 726	JA59311 / DPPE	2.4 / 1
505	700 / 726	JA59311 / DPPE	1.5 / 1
506	719 / 752	AK52468 / JA94882	2 / 1
507	752 / 753	JA94882 / JA10334	1 / 1
508	701 / 726	JA59312 / DPPE	2.3 / 1
509	719 / 752	AK52468 / JA94882	3 / 1
510	751	JA94881	1
511	719 / 751	AK52468 / JA94881	1 / 3
512	719 / 751	AK52468 / JA94881	1 / 1
513	752 / 753	JA94882 / JA10334	0.2 / 1
514	752 / 753	JA94882 / JA10334	2 / 1
515	751 / 753	JA94881 / JA10334	5 / 1
516	726 / 751	DPPE / JA94881	1 / 3
517	751 / 752	JA94881 / JA94882	0.5 / 1

518	726 / 751	DPPE / JA94881	1 / 1
519	719 / 752	AK52468 / JA94882	1 / 1
520	751 / 752	JA94881 / JA94882	5 / 1
521	722 / 751	DOPE / JA94881	1 / 1
522	726 / 752	DPPE / JA94882	1 / 3
523	726 / 752	DPPE / JA94882	1 / 1
524	700 / 726	JA59311 / DPPE	1.9 / 1
525	700 / 726	JA59311 / DPPE	3 / 1
526	701 / 726	JA59312 / DPPE	1.2 / 1
527	752	JA94882	1
528	719 / 751	AK52468 / JA94881	2 / 1
529	722 / 751	DOPE / JA94881	1 / 3
530	751 / 752	JA94881 / JA94882	2 / 1
531	719 / 751	AK52468 / JA94881	3 / 1
532	722 / 751	DOPE / JA94881	1 / 2
533	752 / 753	JA94882 / JA10334	5 / 1

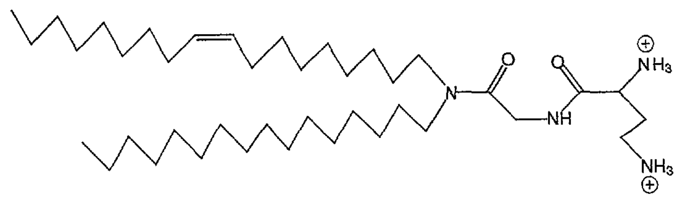
Table X: Lipid Components Names and Structures

Lipid_ID **Name**

700 JA59311

Alternate Names

Palmityloleyl glycyl 2,4-diaminobutyric acid;
POGDABA;
$C_{16}C_{18}GlyDABA^{2+}$;
JA59311

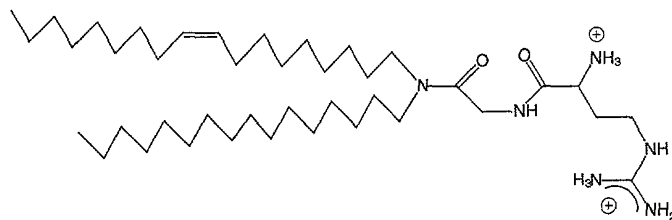


Lipid_ID **Name**

701 JA59312

Alternate Names

JA59312;
Palmityloleyl glycyl 2-amino-4-guanylbutyric acid;
POGAGuaBA;
$C_{16}C_{18}GlyA(Gua)BA^{2+}$

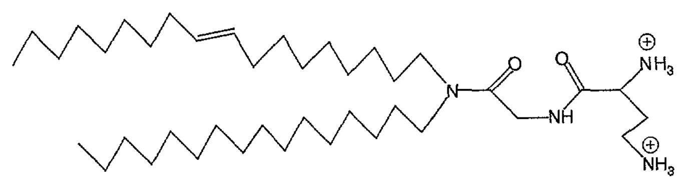


Lipid_ID **Name**

705 JA59396

Alternate Names

N'-Elaidyl-N'-palmityl-a,g-diaminobutyryl-glycinamide;
$C_{16}C_{18:1}GlyDABA^{+2}$;
JA59396

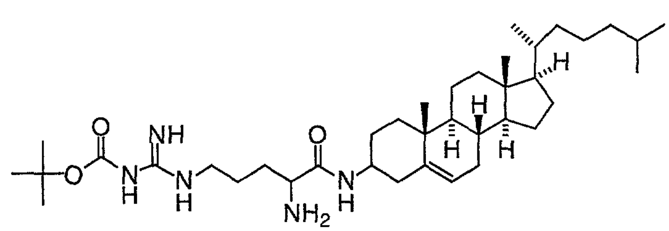


Lipid_ID **Name**

709 DS46596a

Alternate Names

BocArgChol;
DS46596a

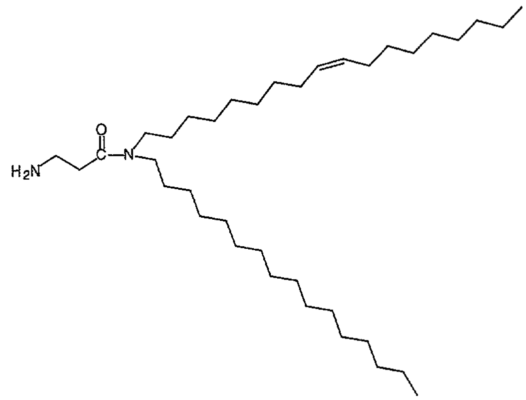


Lipid Components Names and Structures

Lipid_ID **Name**
719 AK52468

Alternate Names

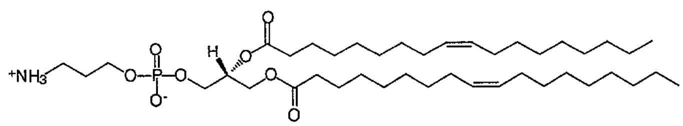
b-Alanine Palmitoyl, Oleoyl-Amide;
POABA;
AK52468



Lipid_ID **Name**
722 DOPE

Alternate Names

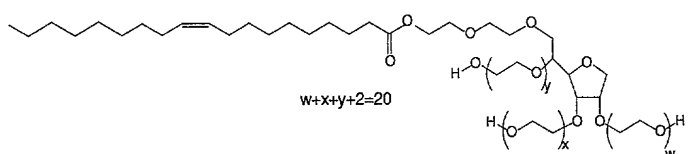
DOPE;
dioleoyl phosphatidyl ethanolamine;
1,2-Dioloeyl-sn-Glycero-3-Phosphatidylethanolamine



Lipid_ID **Name**
723 Tween 80

Alternate Names

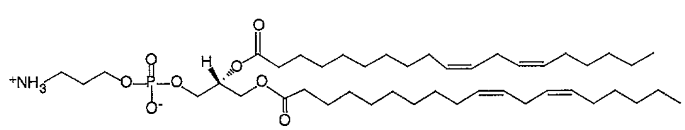
Tween 80;
polyoxyethylene sorbitanmonooleate



Lipid_ID **Name**
725 DLPE

Alternate Names

DLPE;
1,2-Dilinoeyl-sn-Glycero-3-Phosphatidylethanolamine;
Dilinoeyl Phosphatidylethanolamine

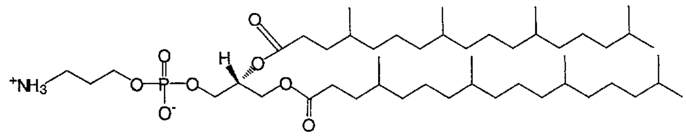


Lipid Components Names and Structures

Lipid_ID	Name
726	DPPE

Alternate Names

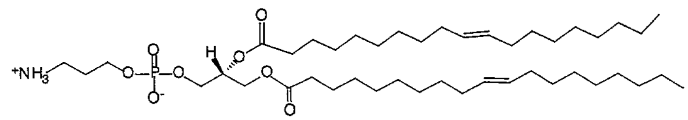
DPPE;
1,2-Diphytanoyl-sn-Glycero-3-Phosphatidylethanolamine;
Diphytanoyl Phosphatidylethanolamine



Lipid_ID	Name
727	DEPE

Alternate Names

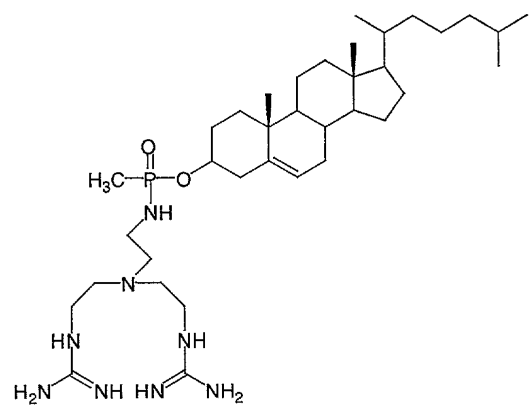
DEPE;
1,2-Dielaidoyl-sn-Glycero-3-Phosphatidylethanolamine;
Dielaidoyl Phosphatidylethanolamine



Lipid_ID	Name
732	PH55942

Alternate Names

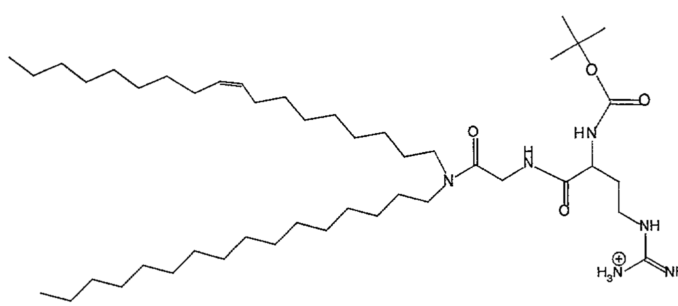
PZH559-42;
Cholesterol-TREN-bis-guanidinium methylphosponamidate



Lipid_ID	Name
736	JA59350

Alternate Names

JA59350;
N'-palmityl-N'-oleyl-N-α-Boc-N-γ-carboxamidine-α,γ-diamin obutyryl-glycinamide



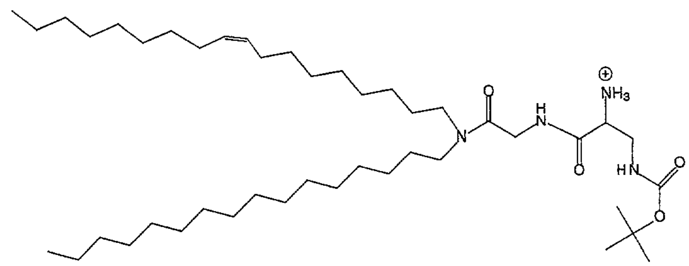
Lipid Components Names and Structures

Lipid_ID **Name**

737 JA59351

Alternate Names

JA59351;
N'-palmityl-N'-oleyl-γ-Boc-α,γ-diaminobutyryl-glycinamide

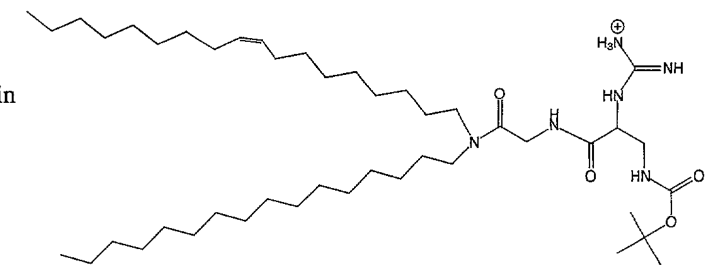


Lipid_ID **Name**

738 JA59352

Alternate Names

JA59352;
N'-palmityl-N'-oleyl-N-γ-Boc-N-α-carboxamidine-α,γ-diamin obutyryl-glycinamide

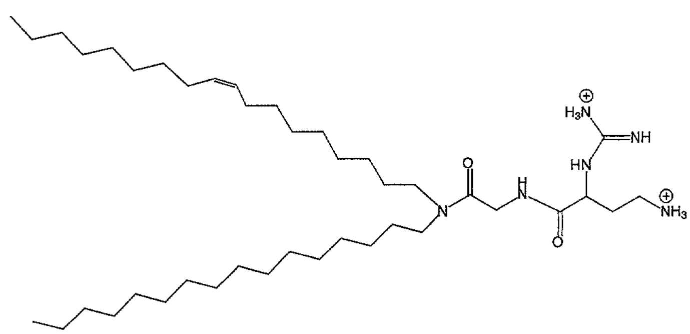


Lipid_ID **Name**

739 JA59353

Alternate Names

JA59353;
N'-palmityl-N'-oleyl-N-γ-carboxamidine-α,γ-diaminobutyryl-glycinamide

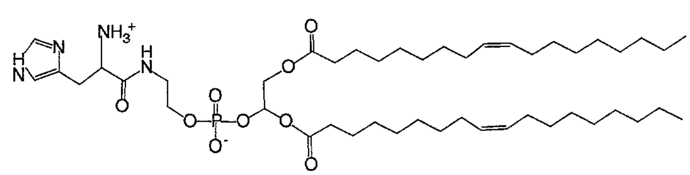


Lipid_ID **Name**

742 JA73851

Alternate Names

N-(L-Histidyl)-L-a-Dioleyl Phosphatidylethanolamine;
His-DOPE



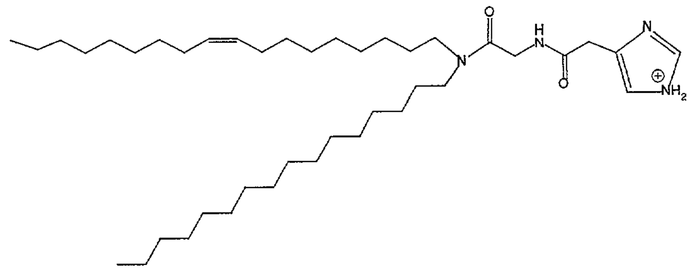
Lipid Components Names and Structures

Lipid_ID **Name**

743 JA73852

Alternate Names

N'-Palmityl-N'-Oleyl-4-Imidazoleacetyl Glycinamide

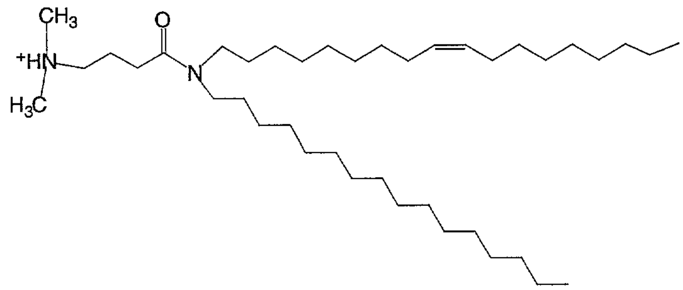


Lipid_ID **Name**

744 JA73853

Alternate Names

N'-Palmityl-N'-Oleyl-4-(dimethylamino)butyrylamide

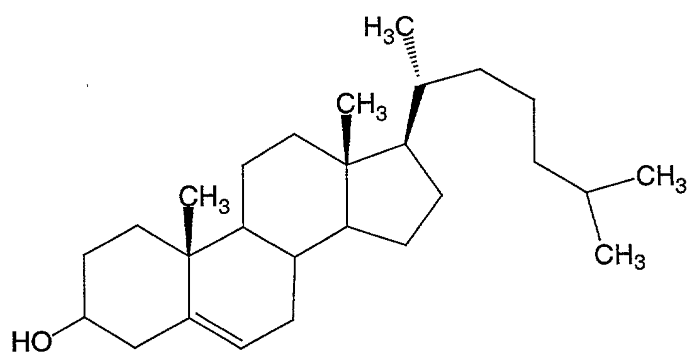


Lipid_ID **Name**

745 cholesterol

Alternate Names

cholesterol;
5-Cholesten-3-beta-ol-3-beta-hydroxy-5-cholestene

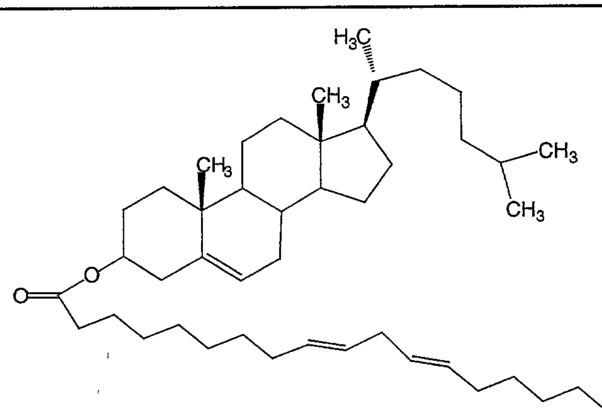


Lipid_ID **Name**

746 chol-linolelaidate

Alternate Names

chol-linolelaidate;
cholesterol-linolelaidate



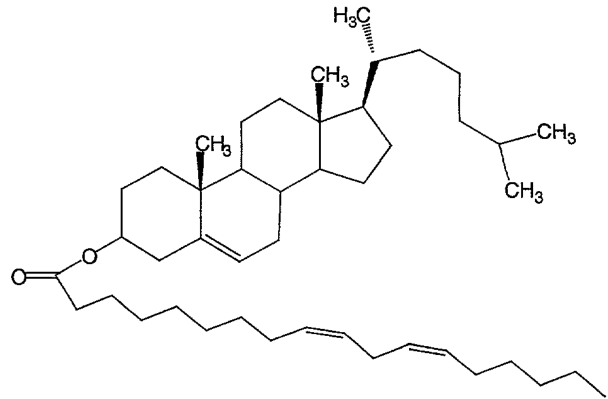
Lipid Components Names and Structures

Lipid_ID **Name**

747 chol-linoleate

Alternate Names

chol-linoleate;
cholesterol-linoleate;
cholesteryl 9,12-octadecadienoate;
5-cholesten-3-beta-ol-3-linoleate

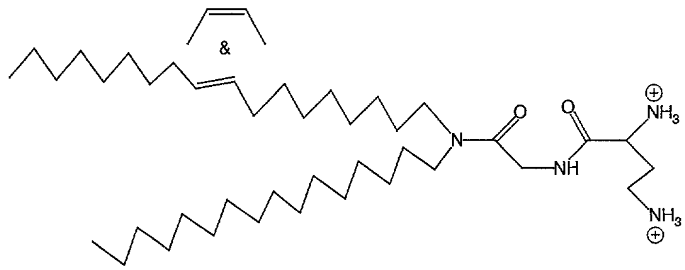


Lipid_ID **Name**

749 EP-G-DABA mix

Alternate Names

N'-Elaidyl-N'-palmityl-α,γ-diaminobutyryl-glycinamide;
$C_{16}C_{18:1}GlyDABA^{+2}$;
EP-G-DABA cis/trans mix

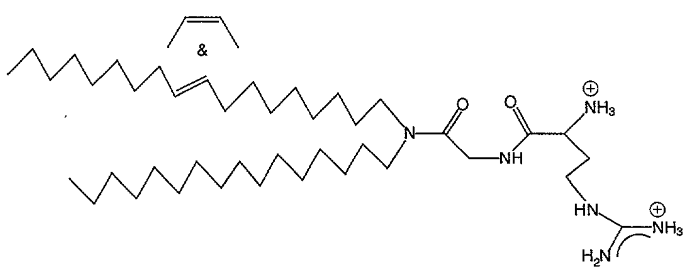


Lipid_ID **Name**

750 EP-G-AGBA mix

Alternate Names

Palmityloleyl glycyl 2-amino-4-guanylbutyric acid;
$C_{16}C_{18:1}GlyA(Gua)BA^{2+}$;
EP-G-AGBA cis/trans mix

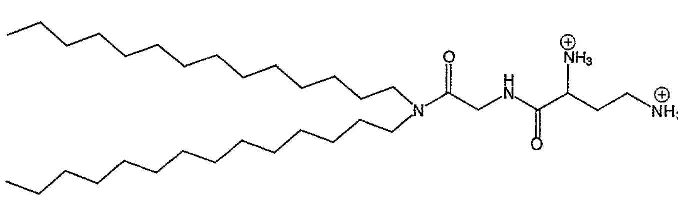


Lipid_ID **Name**

751 JA94881

Alternate Names

N',N'-dimyristyl-α,γ-diaminobutyryl-glycinamide

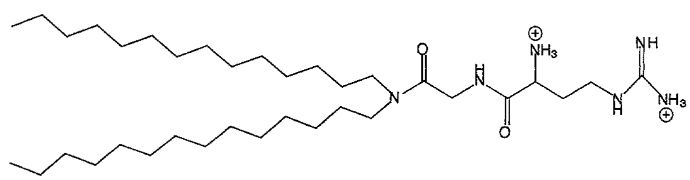


Lipid Components Names and Structures

Lipid_ID	Name
752	JA94882

Alternate Names

N',*N'*-dimirystyl-*N*-γ-carboxamidine-α,γ-diaminobutyryl glycinamide



Lipid_ID	Name
753	JA10334

Alternate Names

N',*N'*-dimirystyl-β-alanylamide